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

A. 510(k) Number:

k173206

B. Purpose for Submission:

New Device

C. Measurand:

Glycosylated Hemoglobin (HbA1c)

D. Type of Test:

Quantitative, enzymatic

E. Applicant:

Sekisui Diagnostics PEI Inc.

F. Proprietary and Established Names:

SEKURE HbA1c Assay

G. Regulatory Information:

Regulatory Description	Classification	Regulation	Product Code	Panel
Hemoglobin A1c Test System	II	21 CFR 862.1373	PDJ	Chemistry, 75
Glycosylated Hemoglobin Assay	II	21 CFR 864.7470	LCP	Hematology, 81

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

The SEKURE HbA1c assay is used to measure the percent concentration of hemoglobin A1c (NGSP) or the HbA1c fraction mmol/mol (IFCC) in human venous whole blood and hemolysate on the SK500 Clinical Chemistry System.
Measurement of hemoglobin A1c is used as an aid in the diagnosis of diabetes mellitus, as an aid in the identification of patients at risk for development of diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.
For In Vitro Diagnostic Use Only

3. Special conditions for use statement(s):
    - For prescription use only
    - For In Vitro Diagnostic Use Only
    - This device has significant negative interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with elevated amounts of HbF including those with known Hereditary Persistence of Fetal Hemoglobin.
    - The HbA1c assay should not be used to diagnose diabetes during pregnancy or in the diagnosis of gestational diabetes. HbA1c reflects the average blood glucose levels over the preceding 3 months (i.e., the average life span of a red blood cell) and therefore may be falsely low during pregnancy or any other condition associated with recent onset of hyperglycemia and/or decreased red blood cell survival.
    - Do not use for testing neonatal blood
    - The HbA1c assay should not be used to diagnose or monitor diabetes in patients with the following conditions:
        - hemoglobinopathies, except as demonstrated to produce acceptable performance (see PERFORMANCE CHARACTERISTICS section of this package insert)
        - abnormal red blood cell turnover (e.g., anemias from hemolysis and iron deficiency)
        - malignancies, and severe chronic hepatic and renal disease
    - In cases of rapidly evolving Type 1 diabetes, the increase of HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions, diabetes mellitus must be diagnosed based on plasma glucose concentrations and/or the typical clinical symptoms.
    - This test should not replace glucose testing for patients with Type 1 diabetes, pediatric patients, or pregnant women.

- Glycated HbF is not detected by the assay as it does not contain the β-chain that characterizes HbA1c. However, HbF is measured in the total Hb assay and as a consequence, specimens containing elevated amounts of HbF may result in lower than expected mmol/mol HbA1c values (IFCC) and %HbA1c values (NGSP).

- The HbA1c assay is susceptible to interference effects from conjugated bilirubin at > 18 mg/dL (216 µmol/L) and unconjugated bilirubin at > 18 mg/dL (307.8 µmol/L).

4. Special instrument requirements:

   Sekisui SK500 Analyzer

I. Device Description:

The SEKURE HbA1c assay is an enzymatic assay for the measurement of the percent hemoglobin A1c concentration. The assay consists of a pre-treatment hemolyzing buffer solution and two working reagents. Testing is performed on the SK500 Analyzer in conjunction with calibrators and controls which are provided separately.

Reagent 1 is a ready for use solution containing 10-(carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)-phenothiazine sodium salt (0.000817%), Protease (Bacterial; < 1 MU/dL), sodium azide (< 0.1 %) as a stabilizer and preservative, and ProClin 300 as a preservative.

Reagent 2 is ready for use and contains Peroxidase (Horseradish; 5 to 15 kU/dL), Fructosyl-peptide-oxidase (E. coli, recombinant; 300 to 900 U/dL), ofloxacin as a preservative. Pre-treatment is a ready for use solution containing Sodium nitrite (> 0.05 to < 0.3%), and ProClin 300 as a preservative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Architect Hemoglobin A1c

2. Predicate 510(k) number(s):

   k130255

3. Comparison with predicate:

Similarities		
Characteristics	**Candidate Device SEKURE HbA1c Assay (k173206)**	**Predicate Device Hemoglobin A1c Assay (k130255)**
Intended Use and Indications for Use	To measure the percent concentration of HbA1c (NGSP) or the HbA1c fraction mmol/mol (IFCC) in human venous whole blood and hemolysate. Measurement of hemoglobin A1c is used as an aid in the diagnosis of diabetes mellitus, as an aid in the identification of patients at risk for development of diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.	Same
Methodology	Enzymatic	Same
Traceability	Traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and Diabetes Control and Complications Trial (DCCT) reference method. Certified via the National Glycohemoglobin Standardization Program (NGSP)	Same
Measuring Interval	4.0 to 14.0% HbA1c (DCCT/NGSP) 20.02 to 129.34 mmol/mol HbA1c (IFCC)	Same

Differences		
Characteristics	**Candidate Device SEKURE HbA1c Assay**	**Predicate Device Hemoglobin A1c Assay (k130255)**
Specimen Type	Whole blood and hemolysate, Dipotassium EDTA, Tripotassium EDTA, Sodium Fluoride/ Disodium EDTA	Whole blood and hemolysate, Dipotassium EDTA, Lithium Heparin, Sodium Heparin, Tripotassium EDTA Sodium Fluoride/Disodium EDTA
Platform	SK500 (k103531) (Clinical Chemistry Analyzer)	ARCHITECT c 8000 (Clinical Chemistry Analyzer)

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

CLSI EP06-A: Evaluation of the Linearity of Quantitative Measuring Procedures: A Statistical Approach; Approved Guideline

CLSI EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition

CLSI EP07-A2: Interference Testing in Clinical Chemistry, Approved Guideline – Second Edition

CLSI EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantification; Approved Guideline – Second Edition

L. Test Principle:

The SEKURE HbA1c assay consists of two separate concentration measurements obtained during a single analysis: Total hemoglobin (THb) and glycated hemoglobin (HbA1c). The two concentrations are used to determine the percent HbA1c (NGSP units) or the hemoglobin fraction in mmol/mol (IFCC units). The individual concentration values of HbA1c and THb generated by the SEKURE HbA1c assay are used only for calculating the percent HbA1c or HbA1c fraction, and must not be used individually for diagnostic purposes.

The SEKURE HbA1c assay is calibrated using a multipoint calibration curve. The calibration is performed using the Blank Solution, Calibrator 1 and Calibrator 2. The Blank solution is used as a zero point calibrator, and contains peroxidase to eliminate noise generated by the reagent. The Blank Solution, Calibrator 1 and Calibrator 2 are available separately.

Pre-treatment: The anticoagulated whole blood sample is lysed using the HbA1c Pre-

treatment solution. The addition of the Pre-treatment solution to the whole blood sample can occur automatically on board the SK500 or may be carried out manually before the sample is placed on board. In the pre-treatment process, the erythrocytes are lysed and the hemoglobin is transformed to methemoglobin by reaction with sodium nitrite.

Total Hemoglobin (THb): The hemoglobin is oxidized to stable methemoglobin azide by the action of sodium nitrite and sodium azide and the concentration of the hemoglobin is determined by bichromatic measurement at 505nm and 800nm.

Glycated Hemoglobin (HbA1c): The SEKURE HbA1c assay utilizes an enzymatic method that specifically measures N-terminal fructosyl dipeptides of the β-chain of HbA1c. With the addition of Reagent 1 to the sample, the glycosylated N- terminal dipeptide (fructosyl-VH) of the β- chain of hemoglobin is released by the action of protease. Following addition of Reagent 2 fructosyl peptideoxidase (FPOX) reacts with fructosyl-VH to produce Hydrogen Peroxide (H_2O_2). The Hydrogen Peroxide reacts with the coloring agent in the presence of Peroxidase (POD) to develop color. The change in absorbance is measured to calculate HbA1c concentration by bichromatic measurement at 660nm and 800nm.

Hemoglobin A1c Calculations: The final result may be expressed as either % HbA1c (NGSP) or mmol/mol HbA1c (IFCC) and is automatically calculated by the system from the HbA1c/THb ratio.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Within-Laboratory Precision (20-Day)

   A 20-day precision study was conducted to evaluate the precision performance of the SEKURE HbA1c assay based on guidance from the CLSI document EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods. The within-lab study was conducted using 3 lots of SEKURE HbA1c reagent on 3 SK500 analyzers and 3 different lots of calibrators for both whole blood and hemolysate applications. The whole blood assay was performed using BioRad Hemoglobin A1c Controls (level 1 and 2 abbreviated below as QC1/QC2) and 4 levels of pooled human K_2 EDTA anticoagulated venous whole blood run in duplicate, twice a day for twenty days. The hemolysate assay included Low and High Controls (abbreviated below as QC1/QC2) as well as 4 levels of pooled human K_2 EDTA anticoagulated venous whole blood run in duplicate, twice a day for twenty days. Each lot of reagent was run on each SK500 for a total of 9 analyzer/lot combinations.

Instrument #1, NGSP Whole Blood

Sample ID	Mean HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	5.22	0.048	0.9	0.033	0.6	0.040	0.8	0.044	0.8	0.070	1.3
QC 2	9.78	0.054	0.6	0.047	0.5	0.050	0.5	0.040	0.4	0.088	0.9
Pool 1	5.00	0.030	0.6	0.040	0.8	0.029	0.6	0.057	1.1	0.058	1.2
Pool 2	6.32	0.033	0.5	0.040	0.6	0.032	0.5	0.061	1.0	0.061	1.0
Pool 3	7.78	0.033	0.4	0.049	0.6	0.027	0.3	0.065	0.8	0.065	0.8
Pool 4	12.05	0.032	0.3	0.051	0.4	0.058	0.5	0.071	0.6	0.084	0.7

Instrument #2, NGSP Whole Blood

Sample ID	Mean HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	5.22	0.057	1.1	0.022	0.4	0.58	1.1	0.040	0.8	0.084	1.6
QC 2	9.77	0.063	0.6	0.024	0.2	0.64	0.7	0.038	0.4	0.093	0.9
Pool 1	5.00	0.054	1.1	0.033	0.7	0.043	0.9	0.051	1.0	0.076	1.5
Pool 2	6.33	0.047	0.7	0.035	0.6	0.054	0.8	0.052	0.8	0.079	1.3
Pool 3	7.79	0.039	0.5	0.037	0.5	0.055	0.7	0.052	0.7	0.076	1.0
Pool 4	12.08	0.032	0.3	0.044	0.4	0.069	0.6	0.056	0.5	0.088	0.7

Instrument #3, NGSP Whole Blood

Sample ID	Mean HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	5.24	0.058	1.1	0.032	0.6	0.067	1.3	0.070	1.3	0.094	1.8
QC 2	9.78	0.062	0.6	0.030	0.3	0.070	0.7	0.045	0.5	0.099	1.0
Pool 1	5.01	0.042	0.8	0.031	0.6	0.050	1.0	0.059	1.2	0.073	1.5
Pool 2	6.34	0.043	0.7	0.037	0.6	0.057	0.9	0.063	1.0	0.080	1.3
Pool 3	7.81	0.040	0.5	0.040	0.5	0.056	0.7	0.061	0.8	0.080	1.0
Pool 4	12.08	0.042	0.3	0.044	0.4	0070	0.6	0.056	0.5	0.092	0.8

Instruments (combined), NGSP Whole Blood

Sample ID	Mean HbA1c %	Repeatability		Between Run		Between Day		Between Instruments		Between Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	5.23	0.055	1.1	0.028	0.5	0.056	1.1	0.053	1.0	0.009	0.2	0.083	1.6
QC 2	9.77	0.064	0.7	0.037	0.4	0.061	0.6	0.041	0.4	0.004	0.0	0.096	1.0
Pool 1	5.01	0.044	0.9	0.034	0.7	0.041	0.8	0.056	1.1	0.007	0.1	0.069	1.4
Pool 2	6.33	0.045	0.7	0.037	0.6	0.047	0.7	0.059	0.9	0.011	0.2	0.074	1.2
Pool 3	7.79	0.037	0.5	0.044	0.6	0.045	0.6	0.059	0.8	0.015	0.2	0.074	0.9
Pool 4	12.07	0.036	0.3	0.049	0.4	0.067	0.6	0.061	0.5	0.021	0.2	0.091	0.8

Instrument #1, NGSP Hemolysate

Sample ID	Mean HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	4.99	0.039	0.8	0.026	0.5	0.025	0.5	0.010	0.2	0.053	1.1
QC 2	9.61	0.034	0.4	0.035	0.4	0.033	0.3	0.014	0.1	0.059	0.6
Pool 1	4.99	0.027	0.5	0.039	0.8	0.025	0.5	0.062	1.2	0.053	1.1
Pool 2	6.32	0.030	0.5	0.040	0.6	0.031	0.5	0.063	1.0	0.059	0.9
Pool 3	7.79	0.020	0.3	0.043	0.6	0.035	0.4	0.063	0.8	0.059	0.8
Pool 4	12.03	0.042	0.3	0.048	0.4	0.054	0.5	0.071	0.6	0.084	0.7

Instrument #2, NGSP Hemolysate

Sample ID	Mean HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	4.98	0.044	0.9	0.031	0.6	0.035	0.7	0.018	0.4	0.064	1.3
QC 2	9.61	0.034	0.3	0.042	0.4	0.028	0.3	0.030	0.3	0.060	0.6
Pool 1	5.00	0.036	0.7	0.023	0.5	0.043	0.9	0.052	1.0	0.060	1.2
Pool 2	6.34	0.036	0.6	0.031	0.5	0.045	0.7	0.050	0.8	0.066	1.0
Pool 3	7.81	0.031	0.4	0.045	0.6	0.048	0.6	0.046	0.6	0.072	0.9
Pool 4	12.06	0.042	0.3	0.042	0.3	0.057	0.5	0.048	0.4	0.083	0.7

Instrument #3, NGSP Hemolysate

Sample ID	Mean HbA1c %	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	5.00	0.055	1.1	0.036	0.7	0.037	0.7	0.017	0.3	0.075	1.5
QC 2	9.62	0.037	0.4	0.045	0.5	0.042	0.4	0.013	0.1	0.072	0.7
Pool 1	5.01	0.034	0.7	0.025	0.5	0.045	0.9	0.064	1.3	0.062	1.2
Pool 2	6.35	0.037	0.6	0.033	0.5	0.05	0.8	0.059	0.9	0.071	1.1
Pool 3	7.82	0.032	0.4	0.059	0.7	0.048	0.6	0.061	0.8	0.082	1.1
Pool 4	12.06	0.052	0.4	0.044	0.4	0.057	0.5	0.052	0.4	0.089	0.7

Instruments (combined) NGSP Hemolysate

Sample ID	Mean HbA1c %	Repeatability		Between Run		Between Day		Between Instruments		Between Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	4.99	0.048	1.0	0.032	0.6	0.035	0.7	0.015	0.3	0.009	0.2	0.067	1.4
QC 2	9.61	0.037	0.4	0.046	0.5	0.036	0.4	0.021	0.2	0.009	0.1	0.069	0.7
Pool 1	5.00	0.033	0.7	0.032	0.6	0.037	0.7	0.059	1.2	0.008	0.2	0.060	1.2
Pool 2	6.34	0.036	0.6	0.036	0.6	0.041	0.6	0.057	0.9	0.011	0.2	0.066	1.0
Pool 3	7.81	0.029	0.4	0.055	0.7	0.037	0.5	0.057	0.7	0.017	0.2	0.073	0.9
Pool 4	12.05	0.048	0.4	0.47	0.4	0.054	0.5	0.058	0.5	0.021	0.2	0.086	0.7

Instrument #1 IFCC Whole Blood

Sample ID	Mean HbA1c Mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	33.57	0.521	1.6	0.362	1.1	0.433	1.3	0.479	1.4	0.768	2.3
QC 2	83.38	0.595	0.7	0.518	0.6	0.548	0.7	0.442	0.5	0.961	1.2
Pool 1	31.12	0.326	1.0	0.438	1.4	0.316	1.0	0.627	2.0	0.631	2.0
Pool 2	45.53	0.357	0.8	0.441	1.0	0.352	0.8	0.669	1.5	0.667	1.5
Pool 3	61.48	0.360	0.6	0.539	0.9	0.293	0.5	0.713	1.2	0.711	1.2
Pool 4	108.17	0.347	0.3	0.552	0.5	0.638	0.6	0.772	0.7	0.913	0.8

Instrument #2 IFCC Whole Blood

Sample ID	Mean HbA1c Mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	33.52	0.622	1.9	0.244	0.7	0.631	1.9	0.432	1.3	0.919	2.7
QC 2	88.28	0.688	0.8	0.260	0.3	0.694	0.8	0.412	0.5	1.011	1.2
Pool 1	31.17	0.587	1.9	0.355	1.1	0.467	1.5	0.559	1.8	0.830	2.7
Pool 2	45.63	0.514	1.1	0.382	0.8	0.586	1.3	0.572	1.3	0.868	1.9
Pool 3	61.68	0.428	0.7	0.400	0.6	0.595	1.0	0.566	0.9	0.835	1.4
Pool 4	108.55	0.350	0.3	0.485	0.4	0.752	0.7	0.610	0.6	0.961	0.9

Instrument #3 IFCC Whole Blood

Sample ID	Mean HbA1c Mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	33.71	0.629	1.9	0.345	1.0	0.736	2.2	0.760	2.3	1.028	3.0
QC 2	83.33	0.679	0.8	0.333	0.4	0.768	0.9	0.495	0.6	1.078	1.3
Pool 1	31.28	0.460	1.5	0.341	1.1	0.550	1.8	0.646	2.1	0.795	2.5
Pool 2	45.76	0.468	1.0	0.402	0.9	0.626	1.4	0.684	1.5	0.879	1.9
Pool 3	61.81	0.433	0.7	0.440	0.7	0.615	1.0	0.662	1.1	0.872	1.4
Pool 4	108.57	0.461	0.4	0.476	0.4	0.761	0.7	0.611	0.6	1.009	0.9

Instruments (combined) IFCC Whole Blood

Sample ID	Mean HbA1c Mmol/ mol	Repeatabilit		Between Run		Between Day		Between Instruments		Between Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	33.60	0.600	1.8	0.302	0.9	0.617	1.8	0.576	1.7	0.101	0.3	0.912	2.7
QC 2	83.33	0.698	0.8	0.407	0.5	0.667	0.8	0.451	0.5	0.048	0.1	1.047	1.3
Pool 1	31.19)	0.482	1.5	0.74	1.2	0.452	1.4	0.612	2.0	0.081	0.3	0.759	2.4
Pool 2	45.64	0.488	1.1	0.406	0.9	0.509	1.1	0.643	1.4	0.118	0.3	0.814	1.8
Pool 3	61.66	0.406	0.7	0.486	0.8	0.495	0.8	0.650	1.1	0.166	0.3	0.804	1.3
Pool 4	108.43	0.394	0.4	0.539	0.5	0.731	0.7	0.669	0.6	0.227	0.2	0.990	0.9

Instrument #1 IFCC Hemolysate

Sample ID	Mean HbA1c Mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	30.98	0.425	1.4	0.289	0.9	0.273	0.9	0.104	0.3	0.582	1.9
QC 2	81.47	0.375	0.5	0.381	0.5	0.365	0.4	0.157	0.2	0.647	0.8
Pool 1	31.04	0.293	0.9	0.421	1.4	0.274	0.9	0.674	2.2	0.581	1.9
Pool 2	45.61	0.328	0.7	0.440	1.0	0.335	0.7	0.689	1.5	0.642	1.4
Pool 3	61.60	0.216	0.4	0.471	0.8	0.379	0.6	0.691	1.1	0.642	1.0
Pool 4	107.93	0.456	0.4	0.530	0.5	0.592	0.5	0.776	0.7	0.916	0.8

Instrument #2 IFCC Hemolysate

Sample ID	Mean HbA1c Mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	30.94	0.476	1.5	0.338	1.1	0.383	1.2	0.197	0.6	0.698	2.3
QC 2	81.56	0.366	0.4	0.457	0.6	0.304	0.4	0.327	0.4	0.660	0.8
Pool 1	31.12	0.398	1.3	0.251	0.8	0.465	1.5	0.572	1.8	0.661	2.1
Pool 2	45.77	0.398	0.9	0.337	0.7	0.497	1.1	0.546	1.2	0.720	1.6
Pool 3	61.83	0.337	0.5	0.491	0.8	0.521	0.8	0.505	0.8	0.791	1.3
Pool 4	108.32	0.460	0.4	0.458	0.4	0.628	0.6	0.524	0.5	0.903	0.8

Instrument #3 IFCC Hemolysate

Sample ID	Mean HbA1c Mmol/mol	Repeatability		Between-Run		Between-Day		Between-Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	31.13	0.601	1.9	0.394	1.3	0.402	1.3	0.181	0.6	0.824	2.6
QC 2	81.67	0.406	0.5	0.496	0.6	0.457	0.6	0.144	0.2	0.787	1.0
Pool 1	31.22	0.370	1.2	0.274	0.9	0.494	1.6	0.696	2.2	0.676	2.2
Pool 2	45.84	0.405	0.9	0.364	0.8	0.551	1.2	0.642	1.4	0.775	1.7
Pool 3	61.96	0.352	0.6	0.642	1.0	0.527	0.9	0.661	1.1	0.902	1.5
Pool 4	108.34	0.570	0.5	0.486	0.4	0.623	0.6	0.567	0.5	0.974	0.9

Instruments (combined) IFCC Hemolysate

Sample ID	Mean HbA1c Mmol /mol	Repeatability		Between Run		Between Day		Between Instruments		Between Lot		Total Precision	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
QC 1	31.02	0.524	1.7	0.351	1.1	0.380	1.2	0.166	0.5	0.104	0.3	0.736	2.4
QC 2	81.57	0.402	0.5	0.500	0.6	0.395	0.5	0.226	0.3	0.100	0.1	0.754	0.9
Pool 1	31.13	0.366	1.2	0.354	1.1	0.409	1.3	0.650	2.1	0.092	0.3	0.653	2.1
Pool 2	45.74	0.398	0.9	0.397	0.9	0.449	1.0	0.629	1.4	0.117	0.3	0.719	1.6
Pool 3	61.80	0.314	0.5	0.604	1.0	0.408	0.7	0.624	1.0	0.182	0.3	0.794	1.3
Pool 4	108.20	0.528	0.5	0.510	0.5	0.593	0.5	0.632	0.6	0.234	0.2	0.944	0.9

b. Linearity/assay reportable range:

Linearity studies were conducted in accordance with CLSI EP06-A. Linearity testing was conducted using human venous whole blood specimens collected in K_2EDTA, two lots of SEKURE HbA1c reagent and calibrators on one SK500 analyzer. A dilution series consisting of 11 levels across the assay range was prepared by mixing

high and low HbA1c K_2EDTA venous whole blood samples. Four replicates were tested at each level. The mean observed %HbA1c value was determined for each intermediate dilution and plotted versus the relative analyte concentration. The following table summarizes the linear regression results obtained from one representative lot generated using both NGSP (%HbA1c) and IFCC units (mmol/mol).

NGSP:

Slope	Intercept	R^2	Concentration Range Tested
1.042	-0.325	0.9983	4.19 – 15.82 % HbA1c

IFCC:

Slope	Intercept	R^2	Concentration Range Tested
1.041	-2.371	0.9976	22.17 – 149.23 mmol/mol HbA1c

The linearity study supports the claimed measuring range of 4.0 to 14.0 %HbA1c (NGSP) or 20.02 to 129.34 mmol/mol HbA1c (IFCC).

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

Traceability:

The SEKURE HbA1c assay is traceable to the International Federation of Clinical Chemistry (IFCC) reference calibrators. The SEKURE HbA1c assay is NGSP certified. The NGSP certification expires in one year. See NGSP website for current certification at http://www.ngsp.org.

The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation NGSP (%) = 0.09148 x IFCC (mmol/mol) + 2.152. HbA1c results are provided to the customers using two different units: NGSP equivalent units (%) and IFCC equivalent units (mmol/mol).

d. *Detection limit:*

Limit of Blank (LoB) and Limit of Detection (LoD) were determined using CLSI Guidance EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. Testing was performed using 5 blank samples for LoB and 5 low concentration samples for LoD. The samples were analyzed in quadruplicate using 2 lots of SEKURE HbA1c reagent on 2 SK500 analyzers. The detection limits are summarized in the tables below:

Limit	%HbA1c	µmol/L A1c	µmol/L tHb
Limit of Blank (LoB)	3.27	0.66	0.2
Limit of Detection (LoD)	3.32	0.75	0.5

e. Analytical specificity:

i.) An interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition to assess endogenous substances that could interfere with the SEKURE HbA1c assay. All interferents were tested at % HbA1c concentrations of ~ 6.5 and 8.0% in replicates of ten. Potential interferents were added individually to aliquots of a K_2 EDTA venous whole blood specimen pool. A control portion of the same matrix was prepared and adjusted for volume with the same solvent used to prepare the interferent stock solution. Significant interference was defined by the sponsor as >5% deviation from the control (aliquot with no interfering substance). Results demonstrated that no significant interference was observed with the following substances up to the listed concentrations:

Analytical Specificity (Endogenous Interferents)

Potential Interferent	**Highest Tested Concentration at which no significant interference was observed**
Conjugated Bilirubin	18 mg/dL
Unconjugated Bilirubin	18 mg/dL
Total Protein	22 g/dL
Triglycerides	3000 mg/dL
Rheumatoid Factor	200 IU/mL
Ascorbic Acid	3.0 mg/dL
Urea	667 mg/dL
Vitamin E	8.6 mg/dL

ii.) A drug interference study was conducted was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition using 2 lots of SEKURE HbA1c reagent. All interferents were tested at % HbA1c concentrations of ~ 6.5 and 8.0% in replicates of ten. Potential interferents were added individually to aliquots of a K_2 EDTA venous whole blood specimen pool. A control portion of the same matrix was prepared and adjusted for volume with the same solvent used to prepare the interferent stock solution. Significant interference was defined by the sponsor as >5% deviation from the control (aliquot with no interfering substance). Results demonstrated that no significant interference was observed with the following substances up to the listed concentrations:

Potential Drug Interferent	Highest Tested Concentration at which no significant interference was observed
Acarbose	50 mg/dL
Acetaminophen	20 mg/dL
Acetylsalicylate	50.8 mg/dL
Atorvastatin	600 µg Eq/L
Captopril	0.5 mg/dL
Chlorpropamide	74.7 mg/dL
Cyanate	65 mg/dL
Furosemide	6.0 mg/dL
Gemfibrozil	7.5 mg/dL
Ibuprofen	50 mg/dL
Insulin	450 µU/mL
Losartan	5 mg/dL
Metformin	5.1 mg/dL
Nicotinic Acid	61 mg/dL
Propranolol	0.2 mg/dL
Repaglinide	60 ng/mL

iii.) A hemoglobin variant interference study was performed according to CLSI EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition using K_2 EDTA venous whole blood samples known to contain common hemoglobin variants (HbA2, HbS, HbC, HbE, HbD, and HbF). The variant samples were obtained from several suppliers including NGSP, commercial vendors, and hospital sites. The %HbA1c concentration was measured using the SEKURE HbA1c assay on an SK500 analyzer and compared to results obtained with an FDA cleared and NGSP certified method that has been demonstrated to be free from interference by the respective hemoglobin variants (Architect c8000 for samples containing HbA2, HbC, HbD, and HbS, Trinity Premier Hb9210 for samples containing HbE and Tosoh Automated Glycohemoglobin Analyzer HLC-723 G8 for samples containing HbF). The numbers and concentrations of hemoglobin variants tested and the range of % HbA1c concentrations in which they were tested are shown below.

The sponsor's definition of non-significant interference was ≤5% mean relative deviation between the SEKURE HbA1c Assay result and the NGSP comparator method.

Hemoglobin Variant	N	Range in % Abnormal Variant	Range in %HbA1c Concentration
HbA2	36	4.1 – 6.2	4.06 – 10.0
HbC	14	27.4 - 38.2	4.81 – 10.46
HbD	25	19.6 – 40.2	4.79 – 12.62
HbE	22	24.0 – 28.0	5.2 – 10.0
HbS	15	28.2 – 43.2	4.24 – 13.38
HbF	25	4.4 – 29.3	5.1 – 12.8

results of the hemoglobin variant study are summarized below:

Hemoglobin Variant	Relative % Difference from Reference Concentration at Low and High Concentrations			
	~ 6.0 % HbA1c (5.5 to 6.5 %HbA1c)		~ 9.0 %HbA1c (7.5 to 10.5 %HbA1c)	
	Relative % Difference	Range	Relative % Difference	Range
HbA2	-2.28	-5.56 to 5.08	-1.28	-2.30 to -0.14
HbC	-1.60	-3.30 to 1.90	-1.39	-2.28 to -0.38
HbD	0.10	-5.69 to 2.91	-1.14	-2.83 to 0.67
HbE	3.41	1.85 to 5.42	3.5	-0.50 to 6.34
HbS	2.28	0.51 to 2.40	0.16	-0.94 to 1.73
HbF	HbF interferes with this assay			

The results demonstrate no significant interference observed for HbA2 (≤6.2%), HbS (≤43.2%), HbC (≤38.2%), HbD (≤40.2%), and HbE (≤28.0%).

The device labeling contains the following prominent boxed warning in the package insert labeling:

"*The SEKURE HbA1c assay has significant negative interference with fetal hemoglobin (HbF). HbA1c results are not valid for patients with elevated level of HbF, including those with known Hereditary Persistence of Fetal Hemoglobin.*"

iv. Hemoglobin Derivatives

A hemoglobin derivative study was performed using potential interferents tested at % HbA1c concentrations of ~ 6.5 and 8.0% in replicates of ten. Potential interferents were added individually to aliquots of a whole blood specimen pool. A control portion of the same matrix was prepared and adjusted for volume with the same solvent used to prepare the interferent stock solution. Significant interference was defined by the sponsor as >5% deviation from the control (aliquot with no interfering substance). Specificity was assessed by comparing test samples containing the potential interferents listed below to reference samples. Results demonstrated that no significant interference was observed with the following substances up to the listed concentrations:

f. *Assay cut-off:*

Potential Interferent	Highest Tested Concentration at which no significant interference (>5%) was observed
Glucose (Labile Hemoglobin)	1000 mg/dL
Aspirin (Acetylated hemoglobin)	50.8 mg/dL
Cyanate (Carbamylated Hemoglobin)	65 mg/dL

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Method comparison testing was performed in accordance with CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. One hundred and thirty (130) human whole blood and hemolysate samples in K_2EDTA with values spanning the assay range of 4.3 to 13.8% HbA1c were tested. Each sample was analyzed internally in replicates of two, using two lots each of SEKURE HbA1c reagent on two SK500 analyzers over 5 operating days. The candidate device results were compared to those obtained from testing at a secondary NGSP reference laboratory on the Tosoh G8 HPLC method. The method comparison was performed using both the hemolysate and whole blood application. The distribution of samples spanning the measuring interval for both whole blood and hemolysate methods are as follows:

Method Comparison Sample Range Summary

Hemoglobin A1c level	Number of Samples	% of Samples Tested
≤ 5%	6	4.6
5 – 6%	11	8.5
6 – 6.5%	34	26.2
6.5 – 7%	33	25.4
> 7%	31	35.3
Total samples	130	100

Deming (weighted) and Passing-Bablok regression analyses were performed for the SEKURE HbA1c versus the comparator method. First replicate analysis for one representative reagent lot is summarized below:

Summary of Method Comparison Results

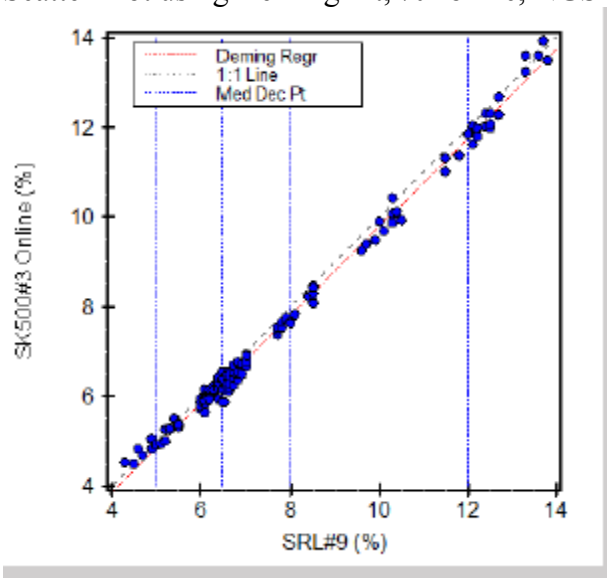
Whole Blood

	Slope	95% CI	y-Intercept	95% CI	R^2
Deming	0.987	0.975-0.999	-0.087	-0.182- 0.007	0.9977
Passing-Bablok	0.974	0.959-0.989	0.007	-0.103-0.115	0.9977

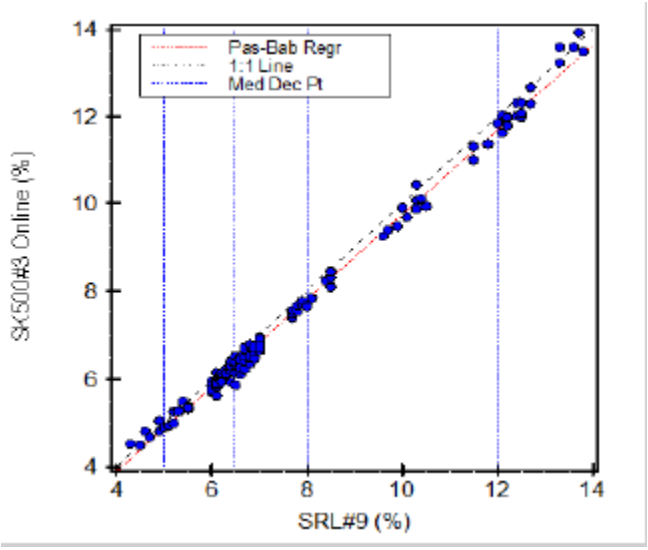
Hemolysate

	Slope	95% CI	y-Intercept	95% CI	R^2
Deming	0.984	0.973-0.995	-0.012	-0.099-0.074	0.9981
Passing-Bablok	0.969	0.955-0.982	0.101	-0.008-0.202	0.9981

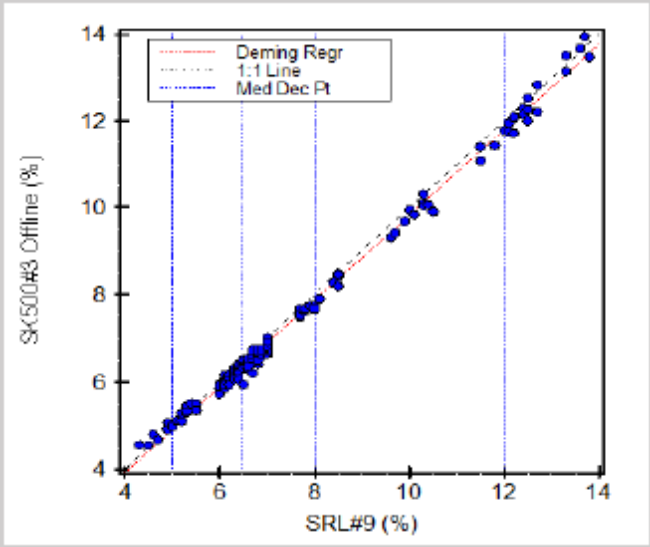
Scatter Plot using Deming Fit, %HbA1c, NGSP vs. SEKURE HbA1c Whole Blood



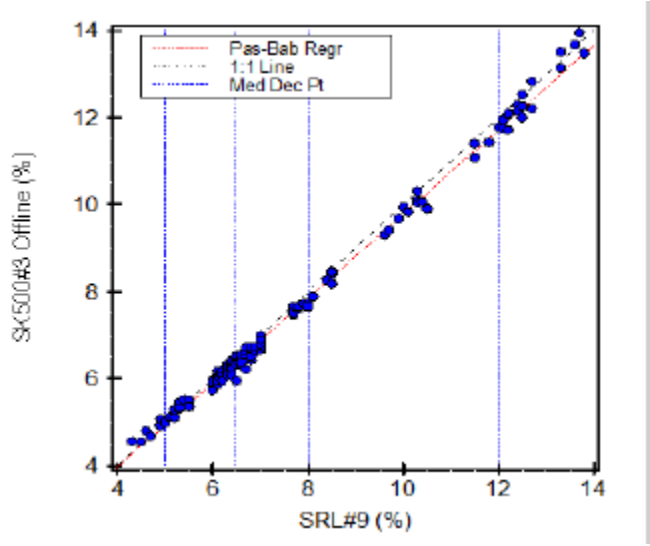
Scatter Plot using Passing-Bablok Fit, %HbA1c, NGSP vs. SEKURE HbA1c Whole Blood



Scatter Plot using Deming Fit, %HbA1c, NGSP vs. SEKURE HbA1c Hemolysate



Scatter Plot using Passing-Bablok Fit, %HbA1c, NGSP vs. SEKURE HbA1c Hemolysate



The following biases between SEKURE HbA1c versus the comparator method were observed:

Bias Estimation table (Passing Bablok)

%HbA1c	Online (Whole Blood)		Offline (Hemolysate)	
	Bias	% Bias	Bias	% Bias
5.00	-0.12	-2.40	-0.06	-1.20
6.50	-0.16	-2.46	-0.10	-1.54
8.00	-0.20	-2.50	-0.15	-1.88
12.00	-0.30	-2.50	-0.28	-2.33

Bias Estimation table (Deming)

%HbA1c	Online (Whole Blood)		Offline (Hemolysate)	
	Bias	% Bias	Bias	% Bias
5.00	-0.15	-3.04	-0.09	-1.84
6.50	-0.17	-2.64	-0.12	-1.78
8.00	-0.19	-2.39	-0.14	-1.75
12.00	-0.24	-2.03	-0.20	-1.70

Total Error Near the Cutoff

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, the Total Error (TE) at the following concentrations (5%, 6.5%, 8% and 12%) was calculated as follows: %TE =|%Bias| + 1.96 * %CV * (1+%Bias/100). The results are presented in the tables below.

% Total Error Summary – Whole Blood (NGSP) Passing Bablok

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-2.40	1.4	5.08
6.5	-2.46	1.2	4.75
8.0	-2.50	0.9	4.22
12.0	-2.50	0.8	4.03

% Total Error Summary – Hemolysate (NGSP) Passing Bablok

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-1.20	1.2	3.52
6.5	-1.54	1.0	3.47
8.0	-1.88	0.9	3.61
12.0	-2.33	0.7	3.67

% Total Error Summary – Whole Blood (NGSP) Deming

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-3.04	1.4	5.70
6.5	-2.64	1.2	4.93
8.0	-2.39	0.9	4.11
12.0	-2.03	0.8	3.56

% Total Error Summary – Hemolysate (NGSP) Deming

% A1c – Decision Level	% Bias	% CV	% TE
5.0	-1.84	1.2	4.15
6.5	-1.78	1.0	3.71
8.0	-1.75	0.9	3.48
12.0	-1.70	0.7	3.05

b. Matrix comparison:

A matrix study was performed to determine the suitability of different anticoagulant collection tube types for use in the SEKURE HbA1c assay using two lots of SEKURE HbA1c reagents on two SK500 analyzers. Matched venous blood specimens were collected from 41 different donors in the control tube type (K_2 EDTA) and in sodium fluoride/K_2 EDTA and K_3 EDTA tube types. Each sample was tested in 2 replicates using two lots of HbA1c reagents. The sample range tested was 5.03-13.69 % HbA1c.

Passing Bablok regression analysis data for one representative lot is provided below.

Sample Type	Slope (95% CI)	Intercept (95% CI)	R2
K_3 EDTA	0.998 (0.984-1.012)	0.062 (-0.025-0.167)	0.9992
Sodium Fluoride/K_2 EDTA	**0.995 (0.983-1.004)**	**-0.021 (-0.082-0.092)**	**0.9994**

The data support the use of the following blood collection tubes with the HbA1c assay:

- K_2 EDTA (control tube)
- Sodium Fluoride/ K_2 EDTA
- K_3 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:

   HbA1c values above 6.5 %HbA1c (48 mmol/mol) meet the criteria for diagnosis of diabetes mellitus.[4] Patients with HbA1c values in the range of 5.7 - 6.4 %HbA1c (39 - 47 mmol/mol) are at an increased risk for diabetes (pre-diabetes).[4] HbA1c levels below 5.7 % HbA1c (39 mmol/mol) are considered normal.[4]

   [4.] American Diabetes Association. Position Statement: Standards of medical care in diabetes – In: Diabetes Care 2018; 41 (Suppl 1): S13-S27.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable and the special controls for this device type under 21 CFR 862.1373.

O. Conclusion:

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