510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K193053

B Applicant

Roche Diagnostics Operations Inc.

C Proprietary and Established Names

Tina-quant Hemoglobin A1cDx Gen.3

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
PDJ	Class II	21 CFR 862.1373 - Hemoglobin A1c Test System	CH - Clinical Chemistry
LCP	Class II	21 CFR 864.7470 - Glycosylated hemoglobin assay	HE - Hematology

II Submission/Device Overview:

A Purpose for Submission:

New device.

B Measurand:

Glycosyclated Hemoglobin (HbA1c)

C Type of Test:

Quantitative turbidimetric inhibition immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Tina-quant Hemoglobin A1cDx Gen.3 assay is intended for use as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes. It is an in vitro diagnostics reagent system intended for quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in hemolysate or venous whole blood on the cobas c 503 clinical chemistry analyzer. HbA1c determinations are useful for monitoring of long-term blood glucose control in individuals with diabetes mellitus.

C Special Conditions for Use Statement(s):

- Rx - For Prescription Use Only
- This device has significant negative interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF including those with known Hereditary Persistence of Fetal Hemoglobin.
- For diagnostic purposes, mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) should be used in conjunction with information from other diagnostic procedures and clinical evaluations.
- The test is designed only for accurate and precise measurement of mmol/mol HbA1c (IFCC) and % HbA1c (DCCT/NGSP). The individual results for total Hb and HbA1c concentration should not be reported.
- 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 high amounts of HbF (>7%) may result in lower than expected mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP).
- As a matter of principle, care must be taken when interpreting any HbA1c result from patients with Hb variants. Abnormal hemoglobins might affect the half life of the red cells or the in vivo glycation rates. In these cases even analytically correct results do not reflect the same level of glycemic control that would be expected in patients with normal hemoglobin. Whenever it is suspected that the presence of an Hb variant (e.g. HbSS, HbCC or HbSC) affects the correlation between the HbA1c value and glycemic control, HbA1c must not be used for the diagnosis of diabetes mellitus.
- Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with a hemoglobinopathy but normal red cell turnover (e.g. sickle cell trait).
- Any cause of shortened erythrocyte survival or decrease in mean erythrocyte age will reduce exposure of erythrocytes to glucose with a consequent decrease in mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP), even though the time-averaged blood glucose level may be elevated. Causes of shortened erythrocyte lifetime might be hemolytic anemia or other hemolytic diseases, homozygous sickle cell trait, pregnancy, recent significant or

chronic blood loss, etc. Similarly, recent blood transfusions can alter the mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP). Caution should be used when interpreting the HbA1c results from patients with these conditions. HbA1c must not be used for the diagnosis of diabetes mellitus in the presence of such conditions.

- Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with hereditary spherocytosis, malignancies or severe chronic hepatic and renal disease.3
- HbA1c should not be used to diagnose diabetes during pregnancy. It reflects the average blood glucose levels over the preceding 3 months (the average life 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 cell survival.
- mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) are not suitable for the diagnosis of gestational diabetes.
- 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 concentration and/or the typical clinical symptoms.
- Hemoglobin A1c testing should not replace glucose testing for type 1 diabetes, in pediatric patients and in pregnant women.

D Special Instrument Requirements:

Roche cobas c 503 Clinical Chemistry Analyzer

IV Device/System Characteristics:

A Device Description:

The Tina-quant Hemoglobin A1cDx Gen.3 assay consists of two working reagents and a Hemolyzing reagent. The R1 reagent consists of antibody reagent, MES buffer: 0.025 mol/L; TRIS buffer: 0.015mol/L, pH6.2; HbA1c antibody (bovine serum): ≥0.5 mg/ml; stabilizers; preservatives (liquid). R2 reagent (Polyhapten reagent) consists of MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2, HbA1c polyhapten: ≥ 8µg/mL; stabilizers; detergents and preservatives (liquid). Anticoagulated whole blood is hemolyzed either manually or automatically prior to determination of HbAlc .

The Tina-quant Hemoglobin A1cDx Gen.3 assay performs separate applications that are specific to the sample types whole blood and hemolysate. The Whole Blood Application differs from the Hemolysate Application in the hemolyzing step. For the Whole Blood Application, whole blood samples are placed on the analyzer and hemolysis occurs onboard the analyzer. For the Hemolysate Application, hemolyzed samples are placed on the analyzer and hemolysis occurs manually before placing the samples onboard the analyzer. The two applications yield the same results.

B Principle of Operation:

HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood. Glycohemoglobin in the sample reacts with anti-HbA1c to form soluble antigen-antibody complexes. Polyhaptens react with excess anti-HbA1c to form an insoluble

antibody-polyhapten complex which can be measured turbidimetrically. The instrument calculates the %HbA1c from the HbA1c/Hb ratio according to a user selected protocol. This method uses tetradecyltrimethylammonium bromide (TTAB) as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary. All hemoglobin variants which are glycated at the β -chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are determined by this assay. Consequently, the metabolic state of patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, HbAE, HbAD) can be determined using this assay.

V Substantial Equivalence Information:

A Predicate Device Name(s):

COBAS INTEGRA 800 Tina –quant HbA1cDx Gen.2 assay

B Predicate 510(k) Number(s):

k121291

C Comparison with Predicate(s):

Device & Predicate Device(s):	K193053	K121291
Device Trade Name	Tina-quant Hemoglobin A1cDx Gen.3	COBAS INTEGRA 800 Tina-quant HbA1cDx Gen.2 assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Same	This test is to be used as an aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes. HbA1c determinations are useful for monitoring of long-term blood glucose control in individuals with diabetes mellitus.
Antibody	Same	Polyclonal anti-HbA1c from sheep blood
General Device Characteristic Differences		
Sample Types	• Li-Heparin • K2-EDTA • K3-EDTA	• Li-Heparin • K2-EDTA • K3-EDTA

Device & Predicate Device(s):	K193053	K121291
	• Fluoride/potassium oxalate • Na-Heparin • EDTA Fluoride	• Fluoride/potassium oxalate • Na-Heparin • NaF/Na2-EDTA

VI Standards/Guidance Documents Referenced:

- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods, 3rd edition
- CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures, 1st edition
- CLSI EP17-A2, Evaluation of Detection Capability of Clinical Laboratory Measurement Procedures, 2nd Edition
- The sponsor satisfied all special controls as outlined in 21 CFR 862.1373.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision studies were conducted to evaluate repeatability (within-run precision) and intermediate precision (within-laboratory precision) according the CLSI guideline EP05-A3. Samples were prepared in K_2EDTA. Two aliquots per sample were measured once each, in two runs per day, for 21 days, on 3 cobas c 503 analyzers and using 3 reagent lots per system. Ten total samples were evaluated in each run: two controls, PreciControl HbA1c norm (Control 1) and PreciControl HbA1c path (Control 2), and eight human samples with approximate Hb1Ac concentrations of 4.9%, 6.6%, 7.3%, 8.2%, 12.5%, 14.6%, 12.3% and 13.1% for venous whole blood and 5.0%, 6.6%, 7.3%, 8.3%, 12.5%, 14.7%, 12.1% and 12.9% for hemolysate applications.

The samples were randomized within each run. For each sample, the following was calculated: mean, repeatability and intermediate precision as CV and SD values and the upper 95% confidence interval for SD and CV values. Results are summarized below:

Hemolysate Application, cobas c 503 Analyzer #1

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-lot		Intermediate Precision (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Hem 1 4.96	0.025	0.5	0.006	0.1	0.071	1.4	0.016	0.3	0.077	1.6
Hem 2 6.62	0.027	0.4	0.013	0.2	0.053	0.8	0.059	0.9	0.085	1.3
Hem 3	0.035	0.5	0.000	0.0	0.053	0.7	0.067	0.9	0.092	1.3

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-lot		Intermediate Precision (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
7.32										
Hem 4 8.32	0.039	0.5	0.009	0.1	0.056	0.7	0.083	1.0	0.108	1.3
Hem 5 12.54	0.057	0.5	0.011	0.1	0.100	0.8	0.203	1.6	0.234	1.9
Hem 6 14.77	0.077	0.5	0.013	0.1	0.148	1.0	0.268	1.8	0.316	2.1
Hem 7 12.14	0.055	0.5	0.023	0.2	0.100	0.8	0.181	1.5	0.215	1.8
Hem 8 12.94	0.072	0.6	0.000	0.0	0.111	0.9	0.188	1.5	0.230	1.8
Control 1 5.53	0.024	0.4	0.009	0.2	0.059	1.1	0.028	0.5	0.071	1.3
Control 2 10.89	0.055	0.5	0.026	0.2	0.085	0.8	0.146	1.3	0.179	1.6

Hemolysate Application, cobas c 503 Analyzer #2

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-lot		Intermediate Precision (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Hem 1 4.96	0.027	0.5	0.005	0.1	0.034	0.7	0.015	0.3	0.046	0.9
Hem 2 6.59	0.035	0.5	0.000	0.0	0.038	0.6	0.057	0.9	0.077	1.2
Hem 3 7.29	0.041	0.6	0.000	0.0	0.043	0.6	0.068	0.9	0.090	1.2
Hem 4 8.28	0.039	0.5	0.015	0.2	0.046	0.6	0.093	1.1	0.112	1.4
Hem 5 12.43	0.069	0.6	0.027	0.2	0.038	0.3	0.175	1.4	0.193	1.6
Hem 6 14.68	0.085	0.6	0.011	0.1	0.060	0.4	0.220	1.5	0.243	1.7
Hem 7 12.05	0.063	0.5	0.018	0.1	0.036	0.3	0.163	1.4	0.179	1.5
Hem 8 12.85	0.071	0.6	0.034	0.3	0.053	0.4	0.177	1.4	0.201	1.6
Control 1 5.52	0.030	0.5	0.008	0.1	0.029	0.5	0.024	0.4	0.049	0.9
Control 2 10.81	0.074	0.7	0.000	0.0	0.041	0.4	0.134	1.2	0.159	1.5

Hemolysate Application, cobas c 503 Analyzer #3

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-lot		Intermediate Precision (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Hem 1 4.94	0.027	0.5	0.010	0.2	0.031	0.6	0.023	0.5	0.048	1.0
Hem 2 6.57	0.030	0.5	0.004	0.1	0.035	0.5	0.049	0.7	0.067	1.0
Hem 3 7.28	0.032	0.4	0.000	0.0	0.038	0.5	0.054	0.7	0.073	1.0
Hem 4 8.28	0.040	0.5	0.000	0.0	0.041	0.5	0.070	0.8	0.091	1.1
Hem 5 12.43	0.063	0.5	0.024	0.2	0.045	0.4	0.162	1.3	0.181	1.5
Hem 6 14.68	0.075	0.5	0.000	0.0	0.050	0.3	0.240	1.6	0.256	1.7
Hem 7 12.07	0.064	0.5	0.000	0.0	0.039	0.3	0.146	1.2	0.164	1.4
Hem 8 12.84	0.072	0.6	0.000	0.0	0.052	0.4	0.150	1.2	0.174	1.4
Control 1 5.49	0.026	0.5	0.008	0.2	0.035	0.6	0.024	0.4	0.050	0.9
Control 2 10.78	0.071	0.7	0.000	0.0	0.048	0.4	0.116	1.1	0.144	1.3

Hemolysate Application, All 3 analyzers (combined)

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Between-Device		Reproducibility (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Hem 1 4.96	0.026	0.5	0.007	0.1	0.049	1.0	0.019	0.4	0.009	0.2	0.059	1.2
Hem 2 6.59	0.031	0.5	0.006	0.1	0.042	0.6	0.055	0.8	0.023	0.3	0.080	1.2
Hem 3 7.30	0.036	0.5	0.000	0.0	0.045	0.6	0.063	0.9	0.019	0.3	0.088	1.2
Hem 4 8.29	0.039	0.5	0.005	0.1	0.049	0.6	0.082	1.0	0.019	0.2	0.105	1.3
Hem 5 12.47	0.063	0.5	0.022	0.2	0.070	0.6	0.179	1.4	0.061	0.5	0.212	1.7
Hem 6 14.71	0.079	0.5	0.010	0.1	0.098	0.7	0.242	1.6	0.053	0.4	0.278	1.9
Hem 7 12.08	0.061	0.5	0.016	0.1	0.067	0.6	0.163	1.3	0.048	0.4	0.193	1.6
Hem 8 12.88	0.072	0.6	0.017	0.1	0.078	0.6	0.172	1.3	0.055	0.4	0.210	1.6

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Between-Device		Reproducibility (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Control 1 5.51	0.027	0.5	0.008	0.2	0.043	0.8	0.027	0.5	0.022	0.4	0.062	1.1
Control 2 10.83	0.067	0.6	0.000	0.0	0.062	0.6	0.132	1.2	0.054	0.5	0.169	1.6

Whole Blood Application, cobas c 503 Analyzer 1

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Intermediate Precision (total)	
	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
WB 1 4.87	0.038	0.8	0.000	0.0	0.043	0.9	0.045	0.9	0.073	1.5
WB 2 6.60	0.026	0.4	0.015	0.2	0.030	0.4	0.071	1.1	0.083	1.3
WB 3 7.37	0.032	0.4	0.018	0.2	0.032	0.4	0.084	1.1	0.097	1.3
WB 4 8.24	0.039	0.5	0.008	0.1	0.048	0.6	0.089	1.1	0.108	1.3
WB 5 12.59	0.056	0.4	0.025	0.2	0.061	0.5	0.154	1.2	0.177	1.4
WB 6 14.69	0.079	0.5	0.043	0.3	0.067	0.5	0.168	1.1	0.202	1.4
WB 7 12.34	0.062	0.5	0.032	0.3	0.057	0.5	0.152	1.2	0.176	1.4
WB 8 13.14	0.061	0.5	0.015	0.1	0.055	0.4	0.165	1.3	0.185	1.4
Control 1 5.51	0.029	0.5	0.002	0.0	0.039	0.7	0.044	0.8	0.066	1.2
Control 2 11.20	0.055	0.5	0.011	0.1	0.057	0.5	0.129	1.2	0.152	1.4

Whole Blood Application, cobas c 503 Analyzer 2

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Intermediate Precision (total)	
	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
WB 1 4.88	0.032	0.7	0.013	0.3	0.026	0.5	0.031	0.6	0.053	1.1
WB 2 6.58	0.031	0.5	0.010	0.2	0.025	0.4	0.077	1.2	0.087	1.3
WB 3 7.35	0.040	0.5	0.000	0.0	0.029	0.4	0.091	1.2	0.103	1.4
WB 4 8.21	0.042	0.5	0.011	0.1	0.035	0.4	0.106	1.3	0.120	1.5
WB 5 12.53	0.070	0.6	0.029	0.2	0.047	0.4	0.164	1.3	0.186	1.5

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Intermediate Precision (total)	
	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
WB 6 14.62	0.095	0.6	0.035	0.2	0.091	0.6	0.198	1.4	0.240	1.6
WB 7 12.25	0.078	0.6	0.000	0.0	0.035	0.3	0.176	1.4	0.195	1.6
WB 8 13.06	0.073	0.6	0.009	0.1	0.052	0.4	0.163	1.2	0.186	1.4
Control 1 5.51	0.036	0.6	0.000	0.0	0.028	0.5	0.044	0.8	0.063	1.1
Control 2 11.13	0.062	0.6	0.033	0.3	0.043	0.4	0.162	1.5	0.182	1.6

Whole Blood Application, cobas c 503 Analyzer 3

Mean % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Intermediate Precision (total)	
	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)	SD	CV(%)
WB 1 4.86	0.029	0.6	0	0.0	0.035	0.7	0.044	0.9	0.064	1.3
WB 2 6.55	0.031	0.5	0.009	0.1	0.025	0.4	0.080	1.2	0.090	1.4
WB 3 7.31	0.039	0.5	0.006	0.1	0.026	0.4	0.090	1.2	0.101	1.4
WB 4 8.17	0.043	0.5	0.012	0.2	0.036	0.4	0.096	1.2	0.111	1.4
WB 5 12.51	0.079	0.6	0.000	0.0	0.064	0.5	0.154	1.2	0.184	1.5
WB 6 14.62	0.082	0.6	0.000	0.0	0.107	0.7	0.191	1.3	0.234	1.6
WB 7 12.23	0.068	0.6	0.028	0.2	0.056	0.5	0.157	1.3	0.182	1.5
WB 8 13.05	0.083	0.6	0.000	0.0	0.064	0.5	0.162	1.2	0.193	1.5
Control 1 5.50	0.030	0.6	0.006	0.1	0.028	0.5	0.051	0.9	0.066	1.2
Control 2 11.10	0.071	0.6	0.000	0.0	0.043	0.4	0.137	1.2	0.160	1.4

Whole Blood Application, All 3 analyzers (combined)

Mean, % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Between-Device		Reproducibility (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
WB 1 4.87	0.034	0.7	0.004	0.1	0.036	0.7	0.040	0.8	0.007	0.1	0.064	1.3

Mean, % HbA1c	Repeatability (error)		Between-Run		Between-Day		Between-Lot		Between-Device		Reproducibility (total)	
	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
WB 2 6.57	0.029	0.4	0.012	0.2	0.028	0.4	0.075	1.1	0.024	0.4	0.090	1.4
WB 3 7.34	0.037	0.5	0.009	0.1	0.032	0.4	0.087	1.2	0.027	0.4	0.104	1.4
WB 4 8.20	0.041	0.5	0.011	0.1	0.042	0.5	0.096	1.2	0.031	0.4	0.117	1.4
WB 5 12.54	0.069	0.5	0.020	0.2	0.062	0.5	0.155	1.2	0.043	0.3	0.187	1.5
WB 6 14.64	0.086	0.6	0.028	0.2	0.096	0.7	0.181	1.2	0.043	0.3	0.228	1.6
WB 7 12.27	0.070	0.6	0.023	0.2	0.055	0.5	0.159	1.3	0.055	0.4	0.192	1.6
WB 8 13.08	0.073	0.6	0.000	0.0	0.061	0.5	0.161	1.2	0.049	0.4	0.194	1.5
Control 1 5.51	0.032	0.6	0.002	0.0	0.032	0.6	0.047	0.8	0.007	0.1	0.065	1.2
Control 2 11.14	0.063	0.6	0.019	0.2	0.054	0.5	0.141	1.3	0.047	0.4	0.171	1.5

2. Linearity:

A linearity study was conducted using one dilution series, consisting of at 12 levels, prepared using a spiked human whole blood pool with a % HbA1c concentration above the upper end of the measuring range. A spiked human whole blood pool, containing purified human HbA0, was used as the diluent; this human whole blood pool had a % HbA1c below the low end of the measuring range. Samples were measured in triplicate, with the Whole Blood Application, and data analysis was performed separately for each sample. Each sample was prepared in K_2EDTA. Results of linear regression analysis were as follows:

Analyte	Slope	Intercept	Pearson's r
%HbA1c	0.95	0.563	0.9981

The linearity study is adequate to demonstrate that the Tina-quant Hemoglobin A1cDx Gen.3 assay is linear from 4.14 % HbA1c to 20.26 % HbA1c. This corresponds to a measuring range of 23-196 mmol/mol HbA1c (IFCC) and 4.2-20.1 % HbA1c (DCCT/NGSP) at a typical hemoglobin concentration of 13.2 g/dL (8.2 mmol/L).

Additionally, a linearity study was conducted according to CLSI EP06-A using two separate dilution series, consisting of at least eleven levels, prepared for each glycated hemoglobin (HbA1c) and total hemoglobin (Hb) using human hemolysate sample pools. The sample pools include HbA1c and Hb concentrations above the upper end of the corresponding measuring range. Each sample was prepared in K_2EDTA, and hemolyzing reagent was used

for the diluent. Samples were measured in triplicate and data analysis was performed separately for each sample.

Samples used to determine linearity spanned the following range:

Analyte	Low end of linear range		High end of linear range	
	g/dL	Mmol/L	g/dL	Mmol/L
Hb	3.04	1.89	40.4	25.1
HbA1c	0.293	0.182	2.87	1.78

Results of linear regression analysis were as follows:

Analyte	Slope	Intercept	Pearson's r
Hb	1.019	-0.1552	0.9999
HbA1c	0.991	-0.0026	0.9990

Results supported device linearity throughout the claimed measuring range of:

Hb: 4 – 40 g/dL (2.48 – 24.8 mmol/L)
HbA1c: 0.3 – 2.6 g/dL (0.186 – 1.61 mmol/L)

3. Analytical Specificity/Interference:

Endogenous Interferences
Studies were performed to evaluate several endogenous substances for potential interference with measurement of % HbA1c using the candidate system. Pooled K_2EDTA whole blood samples, with two hemoglobin A1c levels, one near the medical decision level (approximately 6%) and one above it (approximately 9%), were spiked with the maximum level of the above eight interferents, in separate preparations, resulting in sixteen spiked samples. These samples were then hemolyzed with Tina-quant HbA1c Hemolyzing Reagent. Another pool, without interferent, was equally hemolyzed. A greater than ten-level dilution series was created for each of the sixteen spiked samples, using the interferent-free pools as the diluent.

Each of the 10 dilutions of the sixteen samples were tested in replicates of 10, using one reagent lot, one cobas c 503 analyzer, in a single run and within one calibration cycle. The mean of the ten replicates was compared to the result from the reference sample (aliquot with no interferent). For purposes of this experiment, the data was collected using the Hemolysate Application and was representative of both Hemolysate and Whole Blood Applications. The sponsor defined no significant interference as results that do not demonstrate more than 7% deviation from the reference sample at either % HbA1c levels. The highest concentrations at which no significant interference was observed are summarize below:

Potential Interferent	Highest Tested Concentration without Significant Interference
Bilirubin	60 mg/dL

Potential Interferent	Highest Tested Concentration without Significant Interference
Ditaurobilirubin	60 mg/dL
Triglycerides	1594 mg/dL
Rheumatoid Factors	750 IU/mL
Total Protein	21 g/dL
Albumin	60 g/L
Immunoglobulin (IgG)	60 g/L
Glucose	1000 mg/dL

The labeling states that no significant interference (defined as <7% interference) was observed for the above endogenous interferents at the concentrations listed.

Exogenous Interferences – Drugs

Drug interference testing was performed with hemolysate samples at two different HbA1c levels, approximately 6% and 9% HbA1c. Each drug was added in two defined concentrations with concentration one being several times (typically five times) the maximum daily dosage and concentration 2 being the maximum daily dosage level. Samples were prepared in K_2EDTA and measured in ten-fold using the cobas c 503 analyzer. The median value was compared to the reference value (HbA1c sample with no drug added) and the deviation from the reference was calculated. Interference was defined as median values which were ≤7% deviation from the reference sample. Drug interference studies were conducted using the Hemolysate Application and were representative of both Hemolysate and Whole Blood Applications.

Potential Interferent	Highest Tested Concentration without Significant Interference (mg/dL)
Acetaminophen	20
Acetylsalicylic acid	100
Ampicillin-Na	100
Ascorbic acid	30
Cefoxitin (A)	250
Cefoxitin (B)	660
Cyclosporine	0.5
Doxycyclin	5.0
Gammagard	6000
Heparin	5000 IU/L
Ibuprofen	50

Potential Interferent	Highest Tested Concentration without Significant Interference (mg/dL)
Levodopa	2.0
Methyldopa	2.25
Metronidazole	20
N-Acetylcysteine	166
Phenylbutazone	40
Rifampicin	6.0
Theophylline	10
Tolbutamide	300

No interference was observed at approximately 6% or approximately 9% HbA1c for the drugs evaluated at the concentrations tested. No interference was observed at expected therapeutic concentrations with these drugs. The labeling states that no significant interference (defined as >7% shift in recovery value) was observed for drugs at the concentrations listed in the table above.

Cross-Reactivity

Studies were conducted to evaluate the Tina-quant Hemoglobin A1cDx Gen.3 assay on the cobas c 503 analzyer for potential cross-reactivity with hemoglobin fractions and glycated albumin. Two HbA1c concentrations of pooled whole blood (~6% and ~ 9%) were each mixed with pools of potential cross-reactants, and the % HbA1c values of the samples mixed with interferent pools were compared to the control samples with no interferent. Samples were prepared in K_2EDTA.

Ten replicates of each sample were analyzed using one reagent lot on one cobas c503 analyzer in a single run. For purposes of this experiment, the data was collected using the Hemolysate Application and was representative of both Hemolysate and Whole Blood Applications. The sponsor defined no significant interference as a difference of no more than 7% from the control sample at both % HbA1c levels. The highest concentrations at which no significant interference was observed are summarize below:

Cross reactant	Highest Tested Concentration without Significant Interference
HbA0	120 g/dL
HbA1(a+b)	0.96 g/dL
Carbamylated Hb	2.0 g/dL
Acetylated Hb	2.0 g/dL
Glycated Albumin	10 g/dL
Labile HbA1c	1000 mg/dL

The sponsor's labeling states the following: No cross reactions with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin, glycated albumin and labile HbA1c were found for the anti-HbA1c antibodies used in this kit.

Hemoglobin Variants

Hemoglobin variant testing was conducted to determine if significant interference with any of the major hemoglobin variants occurred when using the Tina-quant Hemoglobin A1cDx Gen.3 assay on cobas c 503 analyzer. The most common hemoglobin variants are HbS, HbC, HbD and HbE. The following is a table of samples that were measured (samples were prepared in K_2EDTA):

Hemoglobin Variant	Number of Samples	% Variant	HbA1c %
HbS	30	35-41 % HbS	4.35 - 12.7
HbC	30	28-37 % HbC	4.90 - 14.1
HbE	30	24-27 % HbE	5.17 - 10.0
HbD	29	36-42 % HbD	5.17 - 9.70
HbA2	15	4.3-6.5 % HbA2	5.10 - 9.80
Elevated HbF	19	3.2-39 % HbF	6.10 -9.30

Each sample was tested once, in on a cobas c 503 analyzer. Results obtained with the Tina-quant Hemoglobin A1cDx Gen.3 assay on the cobas c 503 analyzer were compared to those obtained with the corresponding comparator method. For purposes of this experiment, the data was collected using the Hemolysate Application and was representative of both Hemolysate and Whole Blood Applications. Results were determined as described in the table below:

Hemoglobin Variant	Approximately 6% HbA1c		Approximately 9% HbA1c	
	Relative % difference	Range	Relative % Difference	Range
HbS	-2.5 %	-7.2 – 3.2	-4.0 %	-9.3 – (-2.0)
HbC	-3.9 %	-7.7– 2.8	-6.0 %	-4.6 – (-3.6)
HbE	0.1 %	-5.5 – 5.7	-1.2 %	-5.2 – 0.6
HbD	-1.8 %	-4.5 – 3.0	-2.6 %	-3.3 – 0.2
HbA2	-1.0 %	-4.1– 2.7	0.4 %	-2.2 – 1.1
HbF	Specimens containing high amounts of HbF (>7 %) may yield lower than expected HbA1c values.			

The sponsor has included the following prominent boxed warning in the labeling: "This device has significant negative interference with fetal hemoglobin (HbF). HbA1c results are invalid for patients with abnormal amounts of HbF including those with known Hereditary Persistence of Fetal Hemoglobin. Refer to the Limitations - interference section of this method sheet for details.

4. Assay Reportable Range:

   See Linearity as described in section A.2, above.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Traceability:

   The assigned HbA1c and total hemoglobin values of the cobas c 513 Tina-quant Hemoglobin A1c Gen.3 assay is certified with the National Glycohemoglobin Standardization Program (NGSP). See NGSP website for current certification at http://www.ngsp.org.

   The derived result of the ratio (%) from the NGSP correlation is calculated from the individual quantitative results for total hemoglobin and Hemoglobin A1c (HbA1c). The International Federation of Clinical Chemistry (IFCC) units of mmol/mol are calculated using the Master Equation: IFCC = (NGSP- 2.15) / 91.5. Two different units are provided to the customers: NGSP equivalent units (%) and IFCC equivalents units (mmol/mol).

6. Detection Limit:

   Limits of blank and detection were determined according to EP17-A2. For determination of Limit of Blank (LoB) one analyte free K_2EDTA. sample was measured with three lots in 10-fold determination. Six runs distributed over ≥ 3 days using one cobas c 503 analyzer were performed. In total, 60 measurements were obtained per lot. LoB was defined by the sponsor as the 95th percentile of the 60 measured values. In their design (n=60) the 95th percentile was the average of the 57th and 58th value.

   For determination of the limit of detection (LoD), five unique human samples in K_2EDTA with low-analyte concentrations were measured with three lots of Tina-quant Hemoglobin A1cDx Gen.3 in two-fold determinations. The measurements were performed in six runs, over ≥ three days, on one cobas c 503 analyzer. In total, 60 measurements were obtained per lot. LoD is defined as the concentration, at which there is a 95% probability that a sample contains analyte. Results for LoB and LoD determination are described in the tables below:

Hb LoB		HbA1c LoB	
0.0530 mmol/L	0.085 g/dL	0.0220 mmol/L	0.035 g/dL

Hb LoD		HbA1c LoD	
0.119 mmol/L	0.192 g/dL	0.0437 mmol/L	0.07 g/dL

   These results correspond to a Limit of Blank of 15 mmol/mol (IFCC) and 3.5 % HbA1c (DCCT/NGSP) and a Limit of Detection of 22 mmol/mol (IFCC) and 4.2 % HbA1c (DCCT/NGSP) at a typical hemoglobin concentration of 13.2 g/dL (8.2 mmol/L).

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed to compare the sample results from the candidate method, Hemoglobin A1cDx Gen.3 assay on the cobas c 503 analzyer, to results from a secondary NGSP reference laboratory using the Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh HPLC system), the NGSP method.

The study was conducted with both the Tina-quant Hemoglobin A1cDx Gen.3 Whole Blood Application and Hemolysate Application.

One hundred and seventy-one whole blood samples and one hundred seventy-three hemolysate samples from the secondary NGSP reference laboratory were used in the evaluation. These samples were measured by the secondary NGSP reference laboratory using the Tosoh HPLC system and the Roche Tina-quant Hemoglobin A1cDx Gen.3 test system. The samples were all native and were tested over a 3 day period, with one lot of reagent, on one cobas c 503 analyzer using K_2EDTA as the anti-coagulant. Deming (weighted), Passing-Bablok regression and linear regression analyses were performed for the whole blood application versus the comparator method and the hemolysate application versus the comparator method. The distribution of samples used in the study is summarized below:

% HbA1c	# Samples Tested		% Samples Tested	
	Whole Blood	Hemolysate	Whole Blood	Hemolysate
≤ 5%	6	6	3.5%	3.5%
5-6 %	23	24	13.5%	13.9%
>6 – 6.5%	31	32	18.1%	18.5%
>6.5 – 7%	37	37	21.6%	21.4%
>7 – 8%	25	25	14.6%	14.5%
>8 – 9%	13	13	7.6%	7.5%
>9%	36	36	21.1%	20.8%
Total	171	173	100%	100%

The tables below summarize the bias between the Tina-quant Hemoglobin A1cDx Gen.3 assay and the NGSP Tosoh reference method.

Regression Analysis (whole blood):

Whole Blood	y-intercept	Slope
Deming	-0.190 95% CI: -0.296-(-0.085)	1.019 95% CI: 1.004-1.034
Passing-Bablok	-0.271 95% CI: -0.399-(-0.141)	1.030 95% CI: 1.011-1.050
Linear Regression	-0.0734 95% CI:-0.151-0.004	1.003 95% CI:0.994-1.012

Regression Analysis (hemolysate):

Hemolysate	y-intercept	Slope
Deming	-0.0589 95% CI: -0.046-0.164	0.999 95% CI: 0.984-1.015
Passing-Bablok	-0.0479 95% CI: -0.173-0.060	1.016 95% CI: 1.000-1.034
Linear Regression	0.215 95% CI:0.139-0.292	0.979 95% CI:0.970-0.988

The predicted bias at HbA1c concentrations near clinical decision points, as determined by Passing/Bablok regression, are described below:

% HbA1c	% Bias (Whole Blood)	% Relative Bias (Whole Blood)	% Bias (Hemolysate)	% Relative Bias (Hemolysate)
5%	-0.121	-2.4%	0.031	0.6%
6.5%	-0.076	-1.2%	0.055	0.8%
8%	-0.031	-0.4%	0.078	1.0%
12%	0.089	0.7%	0.142	1.2%

Total Error:

Total error (%TE) was calculated for both the Hemolysate and Whole Blood Applications using the following equation:

$$\%TE = |\%Bias| + 1.96 * \%CV * (1 + \%Bias/100)$$

The %Bias component was generated during the method comparison studies using the Passing-Bablok regression data and is the %Bias shown in the table below. The variance component (%CV) was generated during the precision studies and represents the total reproducibility shown in the table below:

Total Error Summary – Hemolysate

Hemolysate sample	%HbA1c	% Bias	Precision (%CV)	Total Error (%)
1	4.96	0.63	1.2	3.0
2	6.59	0.87	1.2	3.3
3	7.30	0.94	1.2	3.3
4	8.29	1.02	1.3	3.5
5	12.5	1.22	1.7	4.6
6	14.7	1.27	1.9	5.0
7	12.1	1.20	1.6	4.4
8	12.9	1.23	1.6	4.5
Control 1	5.51	0.73	1.1	2.9
Control 2	10.8	1.16	1.6	4.3

Total Error Summary – Whole Blood

Whole Blood Sample	%HbA1c	% Bias	Precision (%CV)	Total Error (%)
1	4.87	-2.56	1.3	5.2
2	6.57	-1.12	1.4	3.8
3	7.34	-0.69	1.4	3.5
4	8.20	-0.30	1.4	3.1
5	12.5	0.84	1.5	3.8
6	14.6	1.15	1.6	4.2
7	12.3	0.79	1.6	3.9
8	13.1	0.93	1.5	3.9
Control 1	5.51	-1.92	1.2	4.3
Control 2	11.1	0.57	1.5	3.6

2. Matrix Comparison:

The effect on Hemoglobin A1c determination, with the Tina-quant Hemoglobin A1cDx Gen.3 assay, in the presence of anticoagulants was determined on the cobas c 503 analytical unit by comparing values obtained from samples drawn into K2-EDTA to results obtained from samples in K3-EDTA, sodium heparin, lithium heparin, NaF/Potassium oxalate, and EDTA/Fluoride.

41 samples of each anticoagulant and 41 half-filled tubes of each anticoagulant were evaluated, with %HbA1c concentrations for the sample sets ranging from approximately 5% to 13%. The filled and corresponding half-filled (double concentrated) sample tubes were from one donor. Matrix comparison studies were conducted using the Hemolysate Application and was representative of both Hemolysate and Whole Blood Applications. The following are results from regression analysis for each matrix (with both full and ½ full tubes):

K_3-EDTA

Full	y = 1.000x + 0.0100	r = 1.000
½ Full	y = 1.005 x – 0.0343	r = 1.000

Na Heparin

Full	y = 1.008x – 0.0547	r = 1.000
½ Full	y = 0.988x + 0.0522	r = 0.999

Li Heparin

Full	y = 1.000x	r = 1.000
½ Full	y = 1.000x – 0.0200	r = 0.999

NaF/Potassium oxalate

Full	y = 1.000x	r = 1.000
½ Full	y = 1.003 x + 0.00472	r = 1.000

EDTA/Fluoride		
Full	y = 1.000x	r = 1.000
½ Full	y = 1.000 + 0.0100	r = 1.000

The results support the use of the Tina-quant Hemoglobin A1cDx Gen.3 assay with samples collected in Li-heparin, K2-EDTA, K3-EDTA, Na-Heparin, EDTA-fluoride and Fluoride/potassium oxalate.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The labeling states the following (note that results reported per the International Federation of Clinical Chemistry, IFCC, are related to results reported per the NGSP as follows: NGSP = 0.915*IFCC+2.15. For additional information see http://www.ngsp.org/ifccngsp.asp):

Protocol 1 (acc. to IFCC): 20-42 mmol/mol HbA1c.[1,2,3,4]

Protocol 2 (% HbA1c acc. to DCCT/NGSP): 4.0-6.0 % HbA1c.

HbA1c levels higher than the upper end of this reference range are an indication of hyperglycemia during the preceding 2 to 3 months or longer. According to the recommendations of the American Diabetes Association values above 48 mmol/mol HbA1c (IFCC) or 6.5 % HbA1c (DCCT/NGSP) are suitable for the diagnosis of diabetes mellitus.32,39 Patients with HbA1c values in the range of 39-46 mmol/mol HbA1c (IFCC) or 5.7-6.4 % HbA1c (DCCT/NGSP) may be at risk of developing diabetes.[5,6]

HbA1c levels may reach 195 mmol/mol (IFCC) or 20 % (DCCT/NGSP) or higher in poorly controlled diabetes. Therapeutic action is suggested at levels above 64 mmol/mol HbA1c (IFCC) or 8 % HbA1c (DCCT/NGSP). Diabetes patients with HbA1c levels below 53 mmol/mol (IFCC) or 7 % (DCCT/NGSP) meet the goal of the American Diabetes Association.[7,8]

HbA1c levels below the established reference range may indicate recent episodes of hypoglycemia, the presence of Hb variants, or shortened lifetime of erythrocytes.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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2. American Diabetes Association Position Statement. Test of Glycemia in Diabetes. Diabetes Care 2004;27(Suppl 1):91-93.

3. Little RR, Rohlfing C, Wiedmeyer HM, et al. The National Glycohemoglobin Standardization Program (NGSP): a five year progress report. Clin Chem 2001;47:1985-1992.

4. Rohlfing CL, Wiedmeyer HM, Little RR, et al. Defining the relationship between plasma glucose and HbA1c:analysis of glucose profiles and HbA1c in the Diabetes Control and Complications Trial. Diabetes Care 2002;25:275-278.

5. International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes. Diabetes Care 2009;32(7):1327-1334.

6. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010;33(1):62-69.

7. Sacks BW, Bruns DE, Goldstein DE, et al. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem 2002;48:436-472.

8. American Diabetes Association. Standards of Medical Care for patients with diabetes mellitus. Diabetes Care [Suppl.] 1995;18(1):8-15.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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