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

A. 510(k) Number:

k121610

B. Purpose for Submission:

Addition of a diagnostic claim to an existing device

C. Measurand:

Whole blood Glycosylated Hemoglobin (HbA1c)

D. Type of Test:

Quantitative turbidimetric inhibition immunoassay

E. Applicant:

Roche Diagnostics Corporation

F. Proprietary and Established Names:

cobas c 501Tina-quant HbA1cDx Gen.3 assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
PDJ	Class II	21 CFR 862.1373	Chemistry, 75

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   This test is to be used as aid in diagnosis of diabetes and as an aid in identifying patients who may be at risk for developing diabetes. The cobas c 501 Tina-quant HbA1cDx Gen.3

assay is an in vitro diagnostics reagent system intended for quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in whole blood on the Roche/Hitachi cobas c 501 clinical chemistry analyzer.

3. Special conditions for use statement(s):

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.

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).

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)

Hemoglobin A1c should not be used in pregnant patients, patients with homozygous sickle cell trait, hemolytic anemia, or other hemolytic diseases, and recent significant or chronic blood loss.

Hemoglobin A1c should not be used to diagnose diabetes mellitus in patients with hereditary spherocytosis, malignancies or severe chronic hepatic and renal disease.

Hemoglobin A1c should not be used in 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.

For prescription use only.

4. Special instrument requirements:

All performance data was conducted using the Roche cobas c 501Analyzer

I. Device Description:

The cobas c 501 Tina-quant HbA1cDx Gen.3 consists of two working reagents (R1 and R2) and an 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).

The cobas c 501 Tina-quant HbA1cDx Gen.3 assay consists of a whole Blood application which uses an automated on-board sample pretreatment with hemolyzing reagent.

Calibrators (Roche Cfas HbA1c) and controls (Roche PreciControl HbA1c norm and path) are recommended for use with this device. The calibrators and controls were previously cleared under 510(k) numbers k052101 and k103099, respectively.

J. Substantial Equivalence Information:

1. Predicate Device name(s):

COBAS INTEGRA 800 Tina-quant HbA1cDx Gen.2 assay

2. Predicate 510(k) number(s)

k121291

3. Comparison with predicate

Similarities and Differences		
Item	Candidate Device cobas c 501 Tina-quant HbA1cDx Gen.3 assay (k121610)	Predicate Device COBAS INTEGRA 800 Tina-quant HbA1cDx Gen.2 assay (k121291)
Intended Use	In vitro test for the quantitative determination of hemoglobin A1c in hemolysate and whole blood on Roche clinical chemistry analyzers.	Same
Sample Types	Li-Heparin, K2-EDTA, K3-EDTA, KF/Na_2-EDTA, Na-Heparin, NaF/K-Oxalate, NaF/Na_2-EDTA	Same
Calibrator	Calibrators (Roche C.f.a.s HbA1c)	Same
Instrument Platform	Roche cobas c 501 analyzer	Roche COBAS INTEGRA 800 analyzer
Controls	Roche PreciControl HbA1c norm and Roche PreciControl HbA1c path	Same
Measuring Range	4.2-20.1 HbA1c%	4.3 -24.8 HbA1c%

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

CLSI EP5-A2, Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline

CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

Anticoagulated whole blood is hemolyzed prior to determination of HbA1c by a turbidimetric inhibition immunoassay (TINIA). Liberated hemoglobin (Hb) in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum and measured biochromatically. The instrument calculates the %HbA1c from the HbA1c/Hb ratio according to a user selected protocol.

This method used 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed by testing 4 levels of HbA1c in anticoagulated venous whole blood patient samples at the following targeted HbA1c values: 5%, 6.5%, 8% and 12%. Additionally 2 controls, PreciControl HbA1c norm and path, were evaluated in this precision study. Precision was evaluated using 3 cobas c 501 chemistry analyzers and 3 lots of reagent on each analyzer. Two aliquots per sample were analyzed twice a day in singlicate for 21 days. All testing was completed within a single calibration cycle for each instrument.

A summary of the results is shown below:

Cobas c 501 Gen.3 Analyzer #1

Mean	Repeatability		Between Run		Between Day		Between Lot		Total	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 5.05%	0.056	1.1	0.022	0.4	0.047	0.9	0.067	1.3	0.102	2.0
Sample2 6.39%	0.062	1.0	0.035	0.5	0.051	0.8	0.095	1.5	0.129	2.0

Sample3 7.87%	0.078	1.0	0.051	0.7	0.087	1.1	0.053	0.7	0.139	1.8
Sample4 11.28%	0.116	1.0	0.000	0.0	0.084	0.7	0.239	2.1	0.278	2.5
Sample5 5.16%	0.062	1.2	0.034	0.7	0.050	1.0	0.077	1.5	0.115	2.2
Sample6 9.41%	0.085	0.9	0.022	0.2	0.060	0.6	0.177	1.9	0.206	2.2

Cobas c 501 Gen.3 Analyzer#2

Mean	**Repeatability**		**Between Run**		**Between Day**		**Between Lot**		**Total**	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 5.08%	0.054	1.1	0.051	1.0	0.024	0.5	0.028	0.5	0.083	1.6
Sample2 6.43%	0.072	1.1	0.055	0.9	0.032	0.5	0.043	0.7	0.105	1.6
Sample3 8.08%	0.081	1.0	0.060	0.7	0.083	1.0	0.021	0.3	0.133	1.6
Sample4 11.45%	0.107	0.9	0.077	0.7	0.076	0.7	0.175	1.5	0.232	2.0
Sample5 5.22%	0.065	1.2	0.054	1.0	0.014	0.3	0.029	0.6	0.090	1.7
Sample6 9.63%	0.096	1.0	0.047	0.5	0.038	0.4	0.078	0.8	0.138	1.4

Cobas c 501 Gen.3 Analyzer #3

Mean	**Repeatability**		**Between Run**		**Between Day**		**Between Lot**		**Total**	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 5.04%	0.062	1.2	0.026	0.5	0.020	0.4	0.075	1.5	0.103	2.0
Sample2 6.38%	0.076	1.2	0.021	0.3	0.034	0.5	0.037	0.6	0.094	1.5
Sample3 8.01%	0.100	1.2	0.055	0.7	0.027	0.3	0.073	0.9	0.138	1.7
Sample4 11.29%	0.112	1.0	0.097	0.9	0.040	0.4	0.036	0.3	0.157	1.4
Sample5 5.18%	0.076	1.5	0.000	0.0	0.029	0.6	0.133	2.6	0.156	3.0
Sample6 9.49%	0.121	1.3	0.044	0.5	0.000	0.0	0.116	1.2	0.174	1.8

Cobas c 501 Gen.3 (combined)

Mean	Repeatability		Between Run		Between Day		Between Lot		Between Instrument		Total	
HbA1c	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample1 5.05%	0.058	1.1	0.036	0.7	0.032	0.6	0.060	1.2	0.000	0.0	0.096	1.9
Sample2 6.40%	0.070	1.1	0.040	0.6	0.040	0.6	0.064	1.0	0.000	0.0	0.110	1.7
Sample3 7.99%	0.087	1.1	0.056	0.7	0.071	0.9	0.053	0.7	0.100	1.3	0.169	2.1
Sample4 11.34%	0.112	1.0	0.067	0.6	0.069	0.6	0.172	1.5	0.000	0.0	0.227	2.0
Sample5 5.19%	0.068	1.3	0.035	0.7	0.034	0.7	0.090	1.7	0.000	0.0	0.123	2.4
Sample6 9.51%	0.102	1.1	0.039	0.4	0.040	0.4	0.130	1.4	0.079	0.8	0.192	2.0

b. Linearity/assay reportable range:

Linearity was evaluated across the measuring range of the device using a whole blood patient sample pool with a high analyte concentration and a diluent pool. Sample pools were mixed in different ratios to obtain a 20-level dilution series with varying concentrations of Hb and HbA1c covering the entire range (low to high) of the assay The sponsor chose to perform 1st and 3rd order polynomial regression analysis on each of the dilution series. The 3rd order regression was found to generate the best linear fit. The linear regression is

Y=correlation coefficient =0.9927

Intercept= -0.019, slope=1.066, r^2=0.995

The linearity study was reviewed and found acceptable. Based on linearity results, the sponsor claims the assay is linear across the reportable measuring range of 4.2-20.2 HbA1c%

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

Traceability

The assigned HbA1c and total hemoglobin values of the cobas c Tina-quant Hemoglobin A1c Gen.3 assay is certified with the National Glycohemoglobin Standardization Program (NGSP). The NGSP certification expires in one year. 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) / 0.092

Two different units are provided to the customers:

NGSP equivalent units (%) and IFCC equivalents units (mmol/mol)

Calibrator and Control materials:

Value assignment for calibrators (Roche C.f.a.s HbA1c) and controls (Roche PreciControl HbA1c norm and PreciControl HbA1c path) that are recommended for use with this device were previously established under 510(k) numbers k052101 and k103099 respectively.

Stability

Stability for calibrators (Roche Cfas HbA1c) and controls (Roche PreciControl HbA1c norm and PreciControl HbA1c path) that are recommended for use with this device were previously established under 510(k) numbers k052101 and k103099 respectively.

d. Detection limit:

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined by assaying an analyte free sample (blank) and five low HbA1c samples according to CLSI guideline EP17A. Each sample was assayed twice a day for three days on two Roche cobas c 501 analyzers using the hemolysate application. The detection limits are summarized in the table below.

Platform/Method	LoB (%A1c)	LoD (%A1c)
Roche c 501 analyzer	2.3%	2.5%

e. Analytical specificity:

i.) Endogenous Interference

Interference studies were performed to assess common or known substances that could interfere with the cobas c 501 Tina-quant HbA1cDx Gen.3 assay. Pooled whole blood was spiked with the maximum level of interferent and the same whole blood pool, without interferent (control), was analyzed and compared to the samples

which contained the interferent. The interfering substances were evaluated at two HbA1c levels (~6.2 and ~8.5% HbA1c). Serial dilutions were performed and samples were analyzed to establish at which point the interferent may affect the assay. No significant interference was defined as the % recovery of ≤ ± 7% of the expected 100% recovery. Results showed that no significant interference was found with the following substances up to the stated concentrations below:

Lipemia	600 mg/dL
Conjugated Bilirubin	60 mg/dL
Unconjugated bilirubin	60 mg/dL
Rheumatoid Factor	750 IU/mL
Glucose	1000 mg/dL
Total Protein	21 g/dL

ii.) Drug interferences were also evaluated. Two HbA1c concentrations of pooled whole blood (~6.3% and ~8.6%) were spiked with the maximum level of potential interference and% HbA1c values were compared to the same sample with no potential interferent present. Significant interference was considered present if the % recovery of HbA1c exceeded ± 7% of the expected 100% recovery. Results showed that no significant interference was found with the following substances up to the stated concentrations below:

Endogenous Substance	Level Tested with No Significant Interference
Acetylcystein	150 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic Acid	300 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U/L
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
Doxycyclin	50 mg/L
Acetylsalicylic Acid	1000 mg/L
Rifampicin	60 mg/L
Cyclosporine	5 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L
Phenylbutazone	400 mg/L

iii.) Cross Reactivity with Hemoglobin Derivatives:

Potential interference from HbA0, HbA1a+b , Acetylated Hb, Carbamylated Hb,

Glycated Albumin, and Labile HbA1c were evaluated. Two HbA1c concentrations of pooled whole blood (~6.3% and~ 8.7%) were spiked with the prepared potential interferent, (HbA0: 12mg/mL, HbA1a+b: 0.16mg/mL, acetylated Hb: 0.2 mg/mL, Carbamylated Hb: 0.2 mg/mL and glycated albumin: 1mg/mL). The % HbA1c values were compared to the same sample with no potential interferent present. Ten replicates of each sample pool and different mixing ratios of the pools were analyzed on the cobas c 501 analyzer. Significant interference was considered present if the % recovery of HbA1c exceeded ± 7% of the expected 100% recovery. The sponsor states that there were no cross reactions at physiologically occurring concentrations with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin, glycated albumin and labile HbA1c.

iv.) Hemoglobin Variant Interference:

A hemoglobin variant interference study was performed using a total of 116 samples known to contain Hemoglobin variants S, C, E, D, A2 and F. Testing of the samples was performed in singlicate on the cobas c 501 analyzer and compared to results obtained by a reference method that has been demonstrated to be free from the hemoglobin interference being tested. The following is a table of samples that were measured:

Hemoglobin Variant	Number of Samples	% Content of Variant in sample	Range in Concentration in % HbA1c
HbS	20	31-42% S	4.6-13.0
HbC	19	33-44% C	4.7-13.0
HbE	20	27-33% E	5.0-9.7
HbD	20	34-42% D	4.8-9.8
HbF	20	2-28% F	5.8-10.1
HbA2	17	4-7% A2	4.9-8.6

Hemoglobin Variant Results Summary

	Percent Relative Bias from Reference Method at Low and High Concentrations of HbA1c Samples	
Hb Variant	~6.0 % HbA1c	~9.0 % HbA1c
C	-3.07	-0.35
S	2.17	3.42
E	-1.58	3.46
D	-2.30	3.35
A2	-5.73	-4.12
F	Bias exceeds -7% when HbF content exceeds + 7% (see below for additional discussion on HbF)	

The results show there is no significant interference for HbS, HbC, HbE, HbD, and HbA2 at the concentrations stated in the table above.

The results show there is significant interference due to the presence of HbF in the sample. The extent of interference is directly proportional to the amount of HbF contained within the sample. The labeling states that, "Specimens containing high amounts of HbF (>7%) may result in lower than expected mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP). No significant interference is observed among specimens tested that contain <7% HbF. A negative bias with HbF is directly proportional in magnitude to the %HbF content. For example, significant interference that produces a negative bias of 7% was observed with specimens containing 7% HbF."

In addition, the device labeling contains the following prominent boxed warning:

"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 package insert for details."

The device contains the following additional warnings:

"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."

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The method comparison study was conducted using 141 variant free samples ranging from 4.7 to 12.2% HbA1c were evaluated using the candidate assay method cobas c 501 Tina-quant HbA1cDx Gen.3 assay method. Samples were tested in singlicate over a 3 day period. The results were compared to testing performed at a secondary NGSP reference laboratory using a cleared HPLC-based HbA1c assay. To support the diagnostic claim, the distribution of samples spanned a concentration around the clinical decision points as follows:

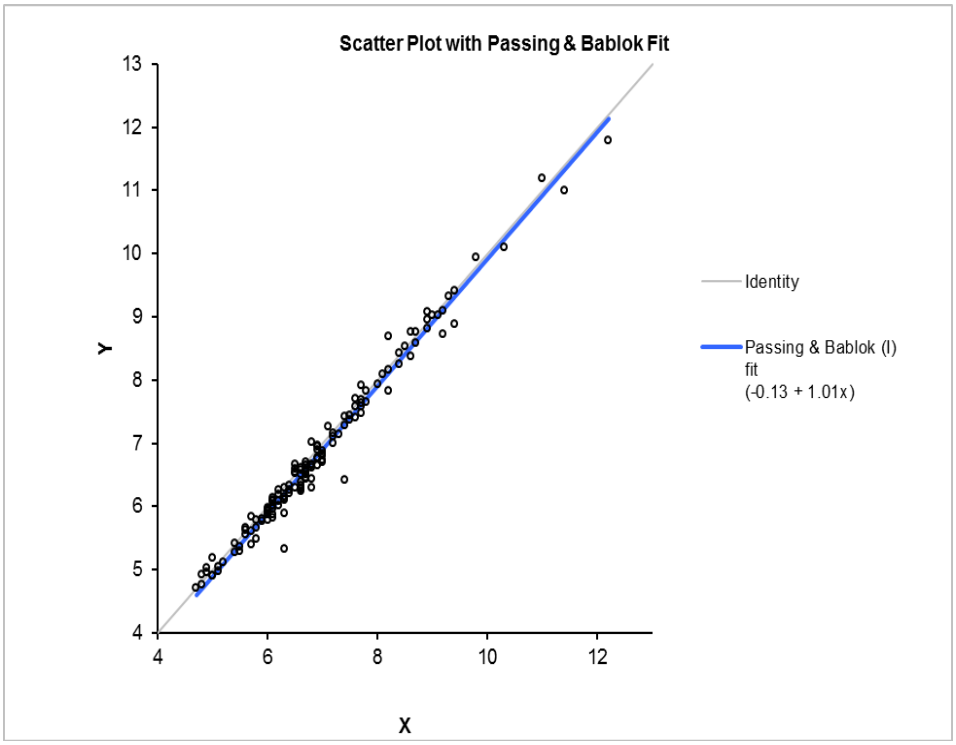
Hemoglobin A1c level	Number of samples	%samples tested
≤ 5%	5	3.5%
5 – 6%	21	14.9%
6 – 6.5%	28	19.9%
6.5 – 7%	33	23.4%
7 – 8%	27	19.1%

8 – 9%	15	10.6%
> 9%	12	8.5%
Total samples	141	100%

Deming (weighted) and Passing-Bablok regression analyses were performed for the Tina-quant HbA1c Gen. 3 (whole blood application) versus the reference method.

Summary of results are as follows:

	y-intercept	Slope
Deming	-0.114 95% CI: -0.301 to 0.072	1.002 95% CI: 0.974 to 1.0285
Passing-Bablok	-0.135 95% CI: -0.297 to 0.010	1.006 95% C: 0.983 to 1.031



The following biases between the cobas c 501 Tina-quant HbA1c Gen.3 assay versus NGSP Tosoh HPLC (Reference Method) were observed:

Decision Level	Bias	%Bias
5.2	-0.103	-1.98
6.5	-0.094	-1.45
8.0	-0.085	-1.06

Total Error Near the Cutoff

Using the results of bias estimation (%Bias) in the method comparison study and precision estimates in the reproducibility study, Total Error (TE) three concentrations: (5.2%, 6.5% and 8.0%) was calculated as follows: %TE =|%Bias| + 1.96 *%CV*(1+%Bias). The results are presented in the tables below.

Decision Level	%Bias	%CV	%TE
5.2	-1.98%	2.07%	6.0%
6.5	-1.45%	1.7%	4.7%
8.0	-1.06%	2.1%	5.1%

b. Matrix comparison:

Acceptable sample types for this assay include Li-Heparin, K2-EDTA, K3-EDTA, KF/Na2-EDTA, Na-Heparin, NaF/K-Oxalate and NaF/Na2-EDTA. Previously established in k102914.

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:

Protocol 1 (acc. to IFCC): 20-42 mmol/mol HbA1c

Protocol 2 (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. 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 a risk of developing diabetes. [1, 2]

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 HbA1c (IFCC) or 7 % HbA1c (DCCT/NGSP) meet the goal of the American Diabetes Association.[3, 4]

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.

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

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

[3] 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.

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

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.