

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

A. 510(k) Number:

k080123

B. Purpose for Submission:

New device

C. Measurand:

Whole blood glucose and glycosylated hemoglobin (HbA1c)

D. Type of Test:

Quantitative Colorimetric glucose and hemoglobin and Turbidimetric HbA1c

E. Applicant:

Wako Chemicals USA, Inc

F. Proprietary and Established Names:

APOLOWAKO GLU
APOLOWAKO HbA1c

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CFR – Glucose test system	Class II	21 CFR 862.1345	75, Chemistry
LCP – Glycosylated hemoglobin assay	Class II	21 CFR 864.7470	81, Hematology

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The APOLOWAKO HbA1c and APOLOWAKO Glucose are for the quantitative determination on the APOLOWAKO analyzer of hemoglobin A1c (HbA1c %) and glucose in whole blood samples.

HbA1c - Measurement of % HbA1c is used to monitor long-term glucose control in individuals with diabetes mellitus.

Glucose - Measurement of glucose is used in the diagnosis and treatment of carbohydrate metabolism disorders, including diabetes mellitus, neonatal hypoglycemia and idiopathic hypoglycemia and pancreatic islet cell carcinoma.

3. Special conditions for use statement(s):

For prescription and point-of-care use

4. Special instrument requirements:

APOLOWAKO Analyzer

I. Device Description:

The APOLOWAKO test system contains the following:

APOLOWAKO Analyzer is a fully contained system, consisting of an automated liquid dispenser, temperature controlled reagent carousel, analysis compartment, sample holder and a display screen. The analyzer uses liquid reagents which are packaged into kits.

APOLOWAKO Glucose test kit contains 2 reagent units. Each unit contains three reagent bottles. Two of the bottles are ready to use liquid reagents and are Enzyme containing good's buffer at pH6.5, hexokinase (HK Yeast), glucose-6-phosphate dehydrogenase (G-6-PGH microorganism), β -nicotinamide adenine dinucleotide oxidized form (NAD yeast) and sodium azide. The liquid calibrator contains 300 mg/dL of glucose and sodium azide.

APOLOWAKO HbA1c test contains one packaged kit which contains 3 reagent units for HbA1c and 2 reagent units for Hemoglobin. HbA1c are liquid ready to use reagents and are 1) anti-HbA1c containing good's buffer at pH of 6.2 and anti-human

HbA1c antibody (sheep, polyclonal), 2) Polyhapten containing good's buffer at pH 6.2 and HbA1c polyhapten and 3) the calibrator contains hemolysate and sodium azide. The Hemoglobin is a liquid ready to use reagent. It includes a hemolyzing reagent containing tetradecyltrimethylammonium bromide and a buffer containing phosphate buffer at pH of 7.4.

All reagents have a reagent information tag on the back of each unit. The information contained on the tag controls the reagent parameters and conditions such as calibration, reagent quantity, shelf-life and lot number.

Other materials required:

APOLOWAKO Color standard, a one package kit containing 2 reagent units. Each unit contains two reagent bottles. The standard is liquid and ready to use. Reagent 1 – Diluent contains sodium chloride and Reagent 2 – Color Solution contains dye. This standard is used to evaluate the accuracy of the on-board pipetting and detection systems.

APOLOWAKO washing solution
APOLOWAKO measurement disk
APOLOWAKO pure water
Quality control material (sold separately).

All human source materials were tested by FDA approved methods and found to be negative for HIV-1, HIV-2, HCV, and HBsAg.

J. Substantial Equivalence Information:

1. Predicate device name(s):

CHOLESTECH LDX, Cholestech Corporation
CHOLESTECH GDX, Cholestech Corporation
Beckman Unicel DxC 800

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

k904082, k011933 and k042291, respectively

3. Comparison with predicate:

Similarities/Differences			
Item	Device	LDX/GDX	Beckman Unicel DxC 800
Indications for use	Measurement of percent HbA1c is used to monitor long-term glucose control in individuals with diabetes mellitus. Measurement of glucose is used in the diagnosis and treatment of carbohydrate metabolism disorders, including diabetes mellitus, neonatal hypoglycemia and idiopathic hypoglycemia	Same	Measurements of HbA1c are accepted as a method to measure long-term glucose control in patients with diabetes mellitus. Elevated levels of HbA1c% suggest the need for more aggressive treatment of glycemia. Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia and idiopathic hypoglycemia.
Sample	Whole blood	Whole blood	Whole blood (HbA1c) and Serum/plasma (glucose)
Testing Environment	Point-of-Care	Point-of-Care	Professional
Reagent format	Liquid	Dry	Liquid
Methodology	Colorimetric, enzyme-based (glucose), absorbance, immune inhibition (HbA1c)	Colorimetric, affinity chromatography	Colorimetric, enzyme based and Absorbance immune inhibition (HbA1c)

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

- FDA Guidance: Format for Traditional and Abbreviated 510(k)s Guidance for Industry and FDA Staff (2005)
- FDA Draft Guidance for 510(k) Submission of Glycohemoglobin (Glycated or Glycosylated) Hemoglobin for IVDs (1991)
- FDA Guidance for Industry In Vitro Diagnostic Glucose Test System; Final (1998)
- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (2004)
- CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004)

L. Test Principle:

Glucose – The analyzer automatically separates the plasma from whole blood for testing. The plasma is separated using centrifugation carried out in a measurement disk on the instrument. The plasma is then transferred to another cell on the measurement disk where the reaction takes place. When a sample is mixed with the enzyme and ATP reagent, the glucose in the sample yields glucose-6-phosphate (G-6-P) and adenosine-5'-diphosphate (ADP). The G-6-P is converted in the presence of NAD. By measuring the increase in absorbance at 340 nm, the glucose concentration in the sample is determined.

HbA1c - A whole blood sample is dispensed onto the measurement disk. The sample is then diluted and hemolyzed. The hemolyzed solution is transferred to another cell on the measurement disk where the reaction takes place. When the sample is mixed with anti-HbA1c reagent a soluble antigen-antibody complex is formed. HbA1c polyhapten binds with excess antibodies and the resulting agglutination complex is measured turbidimetrically. The degree of turbidity is proportional to the concentration of excess anti HbA1c antibody and is inversely proportional to the concentration of HbA1c in the sample. Hemoglobin concentration is determined in a second cell by measuring the absorbance of Hemoglobin diluted with buffer reagent.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Within-run precision was conducted in-house using three heparinized whole blood samples. Samples 1 and 2 were naturally occurring whole blood samples. Sample 3 was a whole blood sample spiked with analyte. Each sample was tested 21 times in one day. Results are presented in the table below:

	Within-run		
	Mean	SD	CV%
Glucose			
Sample 1	56 mg/dL	1.0	1.8
Sample 2	97 mg/dL	0.7	0.7
Sample 3	348 mg/dL	1.6	0.5
HbA1c			
Sample 1	4.8 mg/dL	.07	1.5
Sample 2	7.7 mg/dL	.09	1.2
Sample 3	16.7 mg/dL	.22	1.3

Between-day precision was conducted using two levels of control for glucose and three hemolysate samples. The samples were tested in-house twice a day for 21 days by multiple operators, instruments and reagent lots. Results are presented in the table below:

	Between-day		
	Mean	SD	CV%
Glucose			
Sample 1	72 mg/dL	0.8	1.1
Sample 2	185 mg/dL	1.2	0.7
HbA1c			
Sample 1	4.8 mg/dL	0.7	1.5
Sample 2	7.0 mg/dL	.21	3.0
Sample 3	15.2 mg/dL	.40	2.6

Point-of-Care precision studies:

Precision studies were conducted at three POC sites with 10 operators typically found in these settings. Two control samples were tested once per day, over 33 days on four instruments. The results are presented below:

	Site 1			Site 2			Site 3		
	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV
Glucose									
Level 1	59.8	0.7	1.1	58.1	1.1	1.8	59.0	1.6	2.7
Level 3	357	2.3	0.6	349.4	4.8	1.4	354.9	7.1	2.0
HbA1c									
Level 1	5.2	0.2	4.2	5.2	0.1	2.6	5.0	0.2	4.9
Level 2	10.8	0.3	2.4	10.4	0.2	1.6	10.3	0.3	2.9

b. Linearity/assay reportable range:

The linearity range for each assay was assessed by diluting whole blood samples for each analyte. Sample one for each analyte covered the assay's low range and was diluted with saline to obtain 5 additional samples. The second sample for each analyte was used to cover the assay's high range. A whole blood sample was spiked then diluted with whole blood to obtain additional samples. The dilutions were assayed and the percent recovery was calculated. The measuring range for each assay is Glucose 18-370 mg/dL and HbA1c 3.0%-16.5%. The results are presented below:

Glucose

Measured Conc. mg/dL	Expected Conc. mg/dL	% Recovery
0	0	
19	18	106
36	35	103
54	53	102
71	70	101
90	88	102
90	91	99
193	196	98
299	302	99
398	407	98
The resulting linear regression is $y = 0.979x + 1.4689$ ($R^2 = 0.9999$)		

HbA1c

Measured Conc. mg/dL	Expected Conc. mg/dL	% Recovery
2.9	2.9	100
3.6	3.5	103
4.0	4.1	98

4.8	4.7	102
5.3	5.3	100
5.7	5.9	97
5.9	5.9	100
8.2	8.0	103
10.7	10.1	106
13.3	12.3	108
15.0	14.4	104
16.5	16.5	100
The resulting linear regression is $y=1.0441x - 0.1526$ ($R^2 = 0.998$)		

Spiked Recovery

Unspiked heparinized whole blood samples at three different concentrations for GLU 45, 56 and 186 mg/dL and for HbA1c 3.5, 5.9 and 5.9 % were used. The base samples were assayed to obtain the concentration of glucose and HbA1c in the samples. Increasing amounts of HbA1c coming from a high sample and glucose were added to the samples and were assayed. The concentrations were measured and the percent recovery was calculated. The recovery for glucose ranged from 96-104% and HbA1c from 100-110%.

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

APOLOWAKO HbA1c assay is traceable to the Diabetes control and Complications Trial (DCCT) Method for measurement of HbA1c. HbA1c values are reported according to the National Glycohemoglobin Standardization Program (NGSP) at the DCCT level.

The sponsor has documented traceability to the NGSP's recommended accuracy base for Hgb A1c by performing a direct comparison with a Secondary Reference Laboratory (SRL) using 40 fresh human specimens. NGSP certifications expire after one year.

APOLOWAKO glucose assay is traceable to the NIST SRM917B standard.

The sponsor recommends using commercially available assayed liquid controls..

Real time stability studies have been conducted. Protocols and acceptance criteria were described and found to be acceptable. The manufacturer claims the following expiration date:

When stored unopened at 2-10 °C, the assay reagent is good until the expiration date.

After opening, the assay reagent is good on board for 28 days when stored at 2-10 °C.

d. Detection limit:

The Limit of Blank and the Limit of Detection for each analyte was determined by running a true blank sample and four low samples. Each sample was assayed in 15 replicates in one day. The testing was performed on one instrument by one operator. The detection limits are 0.7 mg/dL glucose and .099% for HbA1c. See the linearity section above for the measuring range of each analyte.

e. Analytical specificity:

Studies were performed to assess common or known substances that could interfere with the each method. Sponsor states that a substance was considered to show no significant interference if the difference between test sample and the blank sample was <10%. Each analyte was found to have no significant interference at the concentration listed below:

	Glucose	HbA1c
	Highest Level Tested with <10% Interference	Highest Level Tested with <10% Interference
Hemoglobin	500 mg/dL	N/A
Bilirubin	50 mg/dL	50 mg/dL
Conjugated bilirubin	40 mg/dL	40 mg/dL
Intrafat	2.0%	2.0%
Ascorbic acid	50 mg/dL	50 mg/dL
EDTA-2NA	0.5%	0.5%
Heparin sodium	0.1%	0.1%
Sodium fluoride	2.0%	N/A

To study the interference from labile A1c on the assay, two heparinized whole blood samples representing normal and diabetic A1c levels were split into aliquots. Each sample was spiked with glucose to various concentrations ranging from 200-1000 mg/dL. The samples were incubated for three hours at 37°C to facilitate formation of labile A1c. The samples were assayed on the APOLOWAKO analyzer. Sponsor states that the potential interferents tested showed <10% interference at the concentrations tested.

The sponsor claimed that Hemoglobin variants such as Hgb S, C, E and Chicago do not affect their device. Their method does not have interference on these Hgb variants according to the literature. Reference: "National

Glycohemoglobin Standardization Program. In Factors that interfere with GHB (HbA1c) Test results.” <http://www.ngsp.org/prog/factors.htm>.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Performance for the APOLOWAKO Glucose and APOLOWAKO HbA1c was evaluated at three Point-of-Care sites and with a total of ten operators. Operators assayed 302 unaltered clinical samples collected with heparin as the anticoagulant over the three sites as well as an additional 86 spiked samples for glucose and 106 leftover samples for HbA1c. The additional samples were included to help cover the assays ranges. The APOLOWAKO System test results were compared to the Beckman UniCel DxC 800 results. Operators were provided instructions from Quick Reference Guide and Package insert. The results are presented below:

	Slope	Intercept	R2	Sample range
APOLOWAKO GLU	0.972	0.469	0.995	18-359 mg/dL
APOLOWAKO HbA1c	1.003	0.226	0.988	4.1-15.7 %

b. *Matrix comparison:*

A Heparin/EDTA comparison test was performed for the APOLOWAKO Glucose and HbA1c assays. Thirty-five paired heparin and EDTA whole blood samples were compared. The correlation is:

	n	Slope	Intercept	r	Device range
Glucose Heparin vs. EDTA	42	1.002	2.3	0.999	18-339 mg/dL
HbA1c Heparin vs. EDTA	45	0.9771	0.1	0.999	4.7-15.8 %

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):

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Reference ranges are provided in the labeling from literature as follows:

Glucose – Serum Fasting Adult 74-100 mg/dL, Child 60-100 mg/dL

HbA1c – Whole blood (EDTA or Heparin) – 4.0-6.0% (NGSP)

Burtis, C.A., Ashwood, E.R., and Bruns, D.E.: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition, Elsevier Saunders.

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.