510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

k080125

B. Purpose for Submission:

New device

C. Measurand:

Whole blood total cholesterol, triglyceride, HDL- cholesterol, LDL-cholesterol (calculated)

D. Type of Test:

Quantitative, colorimetric, enzyme-based

E. Applicant:

Wako Chemicals USA, Inc.

F. Proprietary and Established Names:

APOLOWAKO T-CHO
APOLOWAKO TG
APOLOWAKO HDL-C
APOLOWAKO Analyzer

G. Regulatory Information:

Product Code	Regulation Section	Classification	Panel
CGO- Cholesterol (total) test system	21 CFR 862.1175	Class I, meets Limitations of exemptions 21 CFR 862.9 (c), (4), (9)	75, Chemistry
CDT – Triglyceride test system	21 CFR 862.1705		
LBS – Lipoprotein test system	21 CFR 862. 1475		
JJE – Discrete photometric chemistry analyzer for clinical use	21 CFR 862.2160		

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The APOLOWAKO T-CHO is for the quantitative determination on the APOLOWAKO analyzer of total cholesterol (T-CHO) in whole blood. The APOLOWAKO HDL-C is for the quantitative determination on the APOLOWAKO analyzer of HDL cholesterol in whole blood. The APOLOWAKO TG is for the quantitative determination on the APOLOWAKO analyzer of triglycerides in whole blood. In conjunction with the above values, the APOLOWAKO analyzer calculates LDL cholesterol for triglyceride values up to 400 mg/dL and T-CHO/HDL-C ratio.

   APOLOWAKO Analyzer is a discrete photometric chemistry analyzer for clinical use in both central laboratories and in point of care sites. The device is intended to duplicate manual analytical procedures by automatically performing various steps such as pipetting, mixing and measuring color intensity. This device is intended for use in conjunction with certain materials to measure a variety of analytes of clinical interest in whole blood samples.

   The measurements of total cholesterol, HDL-cholesterol, triglycerides and LDL cholesterol (by calculation for triglycerides up to 400 mg/dL) when used in conjunction with other biochemical markers and coronary risk factors, is useful in the prediction of CHD/CVD risk and the assessment of CHD/CVD severity.

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 carrousel, analysis compartment, sample holder and a display screen. The analyzer uses liquid reagents which are packaged into kits.

APOLOWAKO Total cholesterol test kit contains 2 reagent units. Each unit contains three reagent bottles. Two of the bottles are ready to use liquid reagent and are Enzyme Color A

containing goods buffer at pH 7.0, cholesterol esterase (CHE microorganism) ascorbate oxidase (AOD microorganism) and N-(3-sulopropyl)-3-methoxy-5-methylaniline (HMMPS), and Enzyme Color B containing goods buffer at pH 6.5, cholesterol oxidase (CO microorganism), peroxidase (POD horseradish) and 4-Aminoantipyrine. The third bottle is a lyophilized human serum calibrator and is reconstituted automatically by the instrument during the calibration process.

APOLOWAKO Triglyceride test kit contains 2 reagent units. Each unit contains three reagent bottles. Two of the bottles are ready to use liquid reagent and are Enzyme Color A contains good's buffer at pH7.0, glycerolkinase (GK microorganism), adenosine 5'-triphosphate disodium salt (ATP), glycerol-3-phosphate oxidase (GPO microorganism, catalase (bovine liver), N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HHMPS) and ascorbate oxidase (AOD microorganism and Enzyme Color B contains good's buffer at pH7.1, lipoprotein lipase (LPL microorganism), peroxidase (POD horseradish), 4-aminoantipyrine and sodium azide. The third bottle is a lyophilized human serum calibrator and is reconstituted automatically by the instrument during the calibration process.

APOLOWAKO HDL-cholesterol test kit contains 2 sets of reagent units. Each unit contains three reagent bottles. Two of the bottles are ready to use liquid reagent and are a Pretreatment reagent contains good's buffer at pH7.0, 4-aminoantipyrine (4-AA), peroxidase (POD horseradish), ascorbate oxidase (AOD microorganism) and anti human β-lipoprotein antibody (sheep) and the Enzyme contains good's buffer at pH 7.0, cholesterol esterase (CHE microorganism), Cholesterol oxidase (CO microorganism) and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxy-4-fluoroaniline (FDAOS). The third bottle is a lyophilized human serum calibrator and is reconstituted automatically by the instrument during the calibration process.

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 is a 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

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
   Beckman Unicel DxC 800

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

   k954778 and k042291, respectively

3. Comparison with predicate:

Similarities/Differences			
Item	Device	Cholestech LDX	Beckman Unicel DxC 800
Intended use	The APOLOWAKO Lipid Panel is used for the in vitro quantitative determination of total cholesterol, HDL-cholesterol, LDL-cholesterol (calculated), triglycerides in whole blood and the T-CHOL/HDL-C ratio.	Same	The Unicel DxC 800 System is used for the in vitro determination of total cholesterol, HDL-cholesterol, LDL cholesterol (by calculation) and triglyceride in human serum or plasma.
Sample	Whole blood	Whole blood	Serum or Plasma
Methodology	Colorimetric, enzyme-based	Colorimetric	Colorimetric, enzyme-based
Testing Environment	Point-of-Care	Point-of-Care	Clinical laboratory
Reagent format	Liquid	Dry	Liquid

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

- Format for Traditional and Abbreviated 510(k)s Guidance for Industry and FDA Staff
- Guidance for 510(k)s on Cholesterol Tests for Clinical Laboratory, Physicians; Office Laboratory, and Home Use
- 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:

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.

Total Cholesterol (T-CHO) uses an enzymatic reaction using cholesterol oxidase and cholesterol esterase which in the presence of peroxidase produces a blue pigment which is read colorimetrically. The amount of total cholesterol contained in the sample is proportional to the absorbance of the blue color.

HDL-cholesterol (HDL-C) uses a two step assay. The first step is an antibody inhibition assay which the antibody in the pretreatment reagent binds to lipoproteins (LDL, VDRL and chylomicrons) in the sample. The second step is an enzymatic reaction using cholesterol esterase and cholesterol oxidase in the presence of peroxidase produces a blue color complex which is read colorimetrically. The amount of HDL-C contained in the sample is proportional to the absorbance of the blue color.

Triglyceride (TG) uses a two step assay. The first step is the removal of free glycerol in the sample using glycerolkinase, glycerol-3-phosphate oxidase and a catalase. The second step is an enzymatic reaction which uses lipoprotein lipase, glycerol lipase and glycerol-3-phosphate oxidase which in the presence of peroxidase which produces a blue pigment and is read colorimetrically. The amount of triglyceride contained in the sample is proportional to the absorbance of the blue color.

LDL-cholesterol is a calculation using the Friedewald equation where,

LDL = (T-CHO) – (HDL-C) - (0.2 x TG)

For a TG value >400 no result will report for the LDL-cholesterol.

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 natural samples and sample 3 was a spiked whole blood sample. Each sample was tested 21 times in one day. Results are presented in the table below:

	Within-run		
	Mean	SD	CV%
T-CHO			
Sample 1	122 mg/dL	1.5	1.2
Sample 2	187 mg/dL	1.4	0.7
Sample 3	322 mg/dL	1.7	0.5
TG			
Sample 1	45 mg/dL	1.3	2.9
Sample 2	139 mg/dL	1.9	1.4
Sample 3	325 mg/dL	3.2	1.0
HDL-C			
Sample 1	30.8 mg/dL	0.5	1.6
Sample 2	51.1 mg/dL	0.7	1.4
Sample 3	95.2 mg/dL	0.5	0.5

Between-day precision used two levels of control and a spiked serum pool. Samples1 and 2 were run in house twice a day for 21 days by multiple operators, instruments and reagent lots. Sample 3 was run twice a day for 9 days. Results are presented in the table below:

	Between-day		
	Mean	SD	CV%
T-CHO			
Sample 1	133 mg/dL	2.3	1.7
Sample 2	269 mg/dL	3.4	1.3
TG			
Sample 1	90 mg/dL	1.6	1.8
Sample 2	193 mg/dL	1.7	0.9
Sample 3	576 mg/dL	8.1	1.4
HDL-C			
Sample 1	38.8 mg/dL	0.6	1.6
Sample 2	75.7 mg/dL	0.9	1.1
Sample 3	100.3 mg/dL	0.5	0.5

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			All Sites		
	Mean mg/dL	SD	% CV	Mean mg/dL	SD	% CV	Mean mg/dL	SD	% CV	Mean mg/dL	SD	% CV
TCHO												
Level 1	87.8	1.7	1.9	89.5	3.1	3.5	88.3	2.3	2.6	88.7	2.6	2.9
Level 3	256.2	4.8	1.9	256.7	3.3	1.3	254.8	3.7	1.5	255.9	3.9	1.5
HDL-C												
Level 1	22.3	0.8	3.6	22.5	0.5	2.0	21.5	0.6	2.9	22.07	0.8	3.5
Level 2	71.9	3.8	5.2	77.4	3.5	3.4	67	3.7	5.5	75.3	5.8	8.0
TRIG												
Level 1	97.8	1.3	1.4	93.2	2.9	3.1	96.3	3.1	3.2	95.4	3.3	3.4
Level 3	206.4	2.4	1.2	197.5	6.7	3.4	200.3	6.6	3.3	200.7	6.8	3.4

b. Linearity/assay reportable range:

The measuring ranges of the assays are T-CHO 25-330 mg/dL, TG 30- 625 mg/dL and HDL-C 8-100 mg/dL. The linearity of the T-CHO, TG and HDL-C measurements was demonstrated for each assay by diluting whole blood samples for each analyte to span the range. The samples ranged in concentration for each analyte as follows T-CHO 27-340 mg/dL, TG 20-745 mg/dL and HDL 5.1-105 mg/dL. The samples were assayed and the percent recovery and linear regressions were calculated. The results are presented in the table below:

	Slope	Intercept	R2	% Recovery
APOLOWAKO T-CHO	1.001	0.627	0.9997	98- 104%
APOLOWAKO TG	1.015	0.608	1.000	100-105%
APOLOWAKO HDL-C	0.9808	0.4721	0.9996	96-102%

Recovery

Unspiked heparinized whole blood samples at three different concentrations T-CHO 46, 136 and 288 mg/dL, TG 44, 69 and 348 mg/dL and HDL-C 25, 41 and 47 mg/dL were used. Increasing amounts of cholesterol, triglyceride and HDL were added and samples (spiked and unspiked) were assayed. The concentrations were measured and the percent recovery was calculated for the spiked samples. The recovery for total cholesterol is 95-103%, Triglyceride 100-103% and HDL-cholesterol 98-106%.

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

Cholesterol and HDL Cholesterol were certified by the Cholesterol Reference Method Laboratory Network (CRMLN) as meeting the National Cholesterol Program's

(NCEP) performance criteria for accuracy and precision.

Sponsor recommends using commercially available liquid assayed control materials.

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 1 day**,** 15 replicates. The testing was performed on one instrument by one operator. The detection limits are 1.7 mg/dL for Total Cholesterol, 2.0 mg/dL for Triglyceride and .13 mg/dL for HDL-cholesterol. See linearity above for measuring ranges of each analyte.

e. Analytical specificity:

Studies were performed to assess common or known substances that could interfere with the each method. Whole blood samples were spiked with varies analyte concentrations. Sponsor states that a substance was considered to show no significant interference when the test sample compared to the blank samples is <10%. Each analyte was found to have no significant interference at the concentration listed below:

	T-CHO	TG	HDL-C
	Highest Level Tested with <10% Interference	Highest Level Tested with <10% Interference	Highest Level Tested with <10%Interference
Hemoglobin	500 mg/dL	500 mg/dL	500 mg/dL
Bilirubin	50 mg/dL	50 mg/dL	50 mg/dL
Conjugated bilirubin	40 mg/dL	40 mg/dL	40 mg/dL
Intrafat	1000 mg/dL		1000 mg/dL
Ascorbic acid	50 mg/dL	50 mg/dL	50 mg/dL
EDTA-2NA	0.5%	0.5%	0.5%
Heparin sodium	0.1%	0.1%	0.1%
Free Glycerol		4000	

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The performance for the APOLOWAKO tests was evaluated at three Point-of-Care sites and with a total of ten operators. Operators assayed 302 unaltered clinical

heparinized whole blood samples collected over the three sites as well as an additional 86 spiked samples obtained from Wako Chemical USA Inc. The spiked samples were included to help cover the assays ranges. The ALPOLOWAKO 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	N
APOLOWAKO T-CHO	0.974	4.095	0.994	92-324	388
APOLOWAKO TG	0.999	8.419	0.997	32-625	388
APOLOWAKO HDL-C	0.958	6.32	0.945	23-91	384

b. Matrix comparison:

A Heparin/EDTA comparison test was performed for the APOLOWAKO T-CHO, TG and HDL-C assays. Thirty-five paired heparin and EDTA whole blood samples were compared. The correlation is:

	n	Slope	Intercept	r	Device range
T-CHO Heparin vs. EDTA	39	0.9994	0.1	0.9995	35-296 mg/dL
TG Heparin vs. EDTA	38	0.9916	-0.3	1.000	36-592 mg/dL
HDL-C Heparin vs. EDTA	35	0.9973	0.2	0.999	15.5-73.2 mg/dL

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The cholesterol, triglyceride and HDL reference ranges are obtained from the literature and are presented in the labeling as follows:

Total Cholesterol – Adult:	Desirable	< 200 mg/dL
	Borderline high	200-239 mg/dL
	High	> 240 mg/dL
HDL-C – Serum	Low	<40 mg/dL
	High	≥ 60 mg/dL
Triglyceride -	Normal	< 150 mg/dL
	Borderline high	150-199 mg/dL
	High	200-499 mg/dL
	Very high	≥ 500 mg/dL

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

NIH: Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Final Report Circulation 106:3143-3421 (2002).

N. Instrument Name:

APOLOWAKO Analyzer

O. System Descriptions:

1. Modes of Operation:

The APOLOWAKO analyzer is a fully-integrated POC test system that can perform up to six analytical tests per individual patient sample. The sample is venous whole blood. To perform a test, place a measurement disk in the analysis compartment and the sample in the sample holder. Close the cover and press start, the analyzing process is fully automated. Only one sample is run at a time. The measurement disk is single use and must be replaced with each sample.
All of the reagents have a reagent information tag applied to the back of each bottle which controls the calibration, reagent quantity, shelf-life and lot number.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes ___X_____ or No _____

3. Specimen Identification:

   Automatically assigned by the instrument or can come from an external PC. Only one sample is analyzed at a time.

4. Specimen Sampling and Handling:

   Venous whole blood – EDTA or Heparin

5. Calibration:

   A calibration is performed automatically when a new reagent is placed on the instrument and is good for 28 days. Two calibration methods are used on the instrument; linear method two-point and multi-point. For the two-point linear calibration the calibrator absorbance is compared with the reference absorbance. If the difference is out of the allowable range the reagent is made unusable. The reference absorbance and allowable range are written onto the reagent tag. A new multi-point calibrations curve is plotted based on the measured calibrator absorbance and the calibration curve information on the reagent information tag. Again this information is written onto the reagent tag. The new ranges are now used as the standard curve for reactions on the instrument.

6. Quality Control:

   Internal quality control automatically checks the reagent (reagent tag), dispensing system, measurement disk and processes so that results are not given when any errors occur.

   Two levels of external quality control materials are recommended and laboratories should follow federal, state and local guidelines.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

APOLOWAKO uses the on-board test function "Serum Index" to analyze samples for hemolysis, icterus and/or lipemia. This serum test is a spectrophotometric assay for interferants; it functions using absorbance test results on the plasma from the patient sample.

Serum Index is a routine test run on each sample. A sample exceeding the upper limit of the acceptable range for each interferant is not further analyzed. For example, patient samples hemolyzed to an Hb concentration above the highest non-interfering Hb concentration (500 mg/dL) are then given an error code "R" and analysis on that sample does not proceed. See section 6.5 "Measurement Errors" in the Technical Reference Guide. Three error codes, "R", "Y" and "T", are shown for the "serum information error" for hemoglobin, bilirubin and lipemia (turbidity), respectively.

The wavelengths used are 340 nm, 405 nm and 450 nm. These results are then subjected to on-board analysis by the APOLOWAKO software to determine the concentration of hemoglobin, bilirubin and the degree of turbidity like Triglyceride turbidity.

Note that the highest acceptable extent of each interference state corresponds to the highest amount shown not to interfere. That value is also presented in the package insert. The cut points that generate the error message are:

Lipemia	>1000 mg/dL Triglyceride
Icterus	>50 mg/dL Bilirubin
Hemolysis	>500 mg/dL Hemoglobin

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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