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

A. 510(k) Number:

k062026

B. Purpose for Submission:

Notification of intent to manufacture and market a new device

C. Measurand:

Cholesterol, Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C) and High Density Lipoprotein Cholesterol (HDL-C)

D. Type of Test:

Cholesterol, LDL-C, VLDL-C, and HDL-C is determined enzymatically using cholesterol esterase and cholesterol oxidase.

E. Applicant:

Atherotech, Inc

F. Proprietary and Established Names:

Proprietary – VAP-NT Cholesterol Test
Established - Cholesterol, LDL, VLDL, HDL

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1175 – Cholesterol
   21 CFR 862.1475 LDL-C, VLDL-C and HDL-C

2. Classification:

   Class I, meets the limitation to the exemption (21 CFR 862.9 (c) (4))

3. Product code:

   CHH, MRR, LBS

4. Panel:

   75, Chemistry

H. Intended Use:

1. Intended use(s):

   See Indications for Use below

2. Indication(s) for use:

   The VAP-NT Cholesterol test is a device intended to measure total cholesterol and its component lipoprotein fractions from VLDL, LDL and HDL, in fasting serum. Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipid metabolism disorders.

3. Special conditions for use statement(s):

   For in vitro diagnostic use only

   For professional use only

4. Special instrument requirements:

   Ultracentrifuge, Spectrophotometer

I. Device Description:

The VAP- NT Cholesterol Test is an in vitro diagnostic medical device used in conjunction with laboratory equipment. The VAP-NT Cholesterol Test is intended to measure total cholesterol, and its component lipoprotein fractions VLDL, LDL and HDL, in fasting serum.

Test Methodology
The VAP-NT Test method consists of a sample preparation phase in which the serum is mixed with potassium bromide (KBr) to adjust the density and layered beneath a saline solution. The sample is spun using an ultracentrifuge and vertical rotor to obtain a density-gradient.

The centrifuge tube is placed into a fluid handling fixture which drains the tube from the bottom and uses piston pumps to control the flow rates. The centrifuged specimen is drained at a defined rate and mixed with a commercially available reagent (Roche CH). The sample is mixed with reagent in a continuous flow by drawing each fluid alternately through a "y" shaped connector. The sample/reagent is heated as it flows through the temperature bath. The reacted specimen is delivered in a continuous flow process to the spectrometer flow cells for absorbance measurements.

A cholesterol profile (absorbance curve) is obtained by plotting the individual absorbance measurements on the Y-coordinate and their relative position in the density gradient on the X-coordinate. The relative position is calculated from the sample drain time.

The VAP-NT software algorithm mathematically deconvolutes the absorbance curve into major lipoprotein curves. The area under each curve is directly proportional to the cholesterol value (i.e. mg/dl). Total Cholesterol is determined as a sum of the area under all subcurves.

The VAP-NT algorithm is based on the premise that the curve shape for individual lipoprotein class and subclass is generally consistent. These fixed subcurve peak parameters are analogous to the use of fixed density ranges for the separation of each lipoprotein class in the ß quantification method and electrophoretic methods.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Quantimetrix Lipoprint, Roche Diagnostics Reagent

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

   Quantimetrix Lipoprint k010337 & k013662
   Roche Diagnostics Reagent k952127

3. Comparison with predicate:

   Substantial Equivalency Comparison- Similarities and Differences

Parameter	**VAP-NT Cholesterol Test**	**Lipoprint**	**Roche Cholesterol Reagent**
510k Number	pending	k010337 and k013662	k952127
Device Name	VAP-NT Cholesterol Test	LipoPrint LDL and HDL	Cholesterol Assay
Intended Use	The VAP cholesterol test is a device intended to measure total cholesterol, and its component lipoprotein fractions ,VLDL, LDL and HDL, in fasting serum. Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders	Intended to measure lipoprotein cholesterol (for lipoprotein fractions and subfractions from VLDL to HDL) in fasting serum or plasma with a Total Cholesterol concentration of $\geq$ 100 mg/dl. Lipoprotein cholesterol measurements are use as an aid in evaluating lipid metabolism disorders when used in conjunction with other lipid tests, patient risk	Enzymatic in vitro test for the direct quantitative determination of cholesterol in human serum and plasma on automated clinical chemistry analyzers

Parameter	VAP-NT Cholesterol Test	Lipoprint	Roche Cholesterol Reagent
		assessment and clinical evaluation	
Measurand	Total Cholesterol LDL Cholesterol, HDL Cholesterol, VLDL Cholesterol	LDL Cholesterol, HDL Cholesterol, VLDL Cholesterol	Total Cholesterol
Methodology	Multi-step procedure that combines separation by ultracentrifugation, colorimetric enzyme based determination of cholesterol and spectrophotometric characterization of the reaction.	Electrophoretic separation	Colorimetric- enzyme based
Testing Environment	Clinical Laboratory	Clinical Laboratory	Clinical Laboratory
Reporting Units	mg/dl	mg/dl	mg/dl
Sample Type	Serum	Serum or plasma	Serum or plasma
Controls/Calibration	Labeling recommends Calibration/ Control materials and procedures	Labeling recommends Calibration/ Control materials and procedures	Labeling recommends Calibration/ Control materials and procedures
Reporting Values	Numeric Values Peak chart	Numeric Values Peak chart	Analyzer dependant
Specimen dilution	Requires mixture (dilution) of sample with potassium bromide and saline.	Requires mixture (dilution) of sample with loading gel.	Allows for dilution and dilution factor to obtain results
Parameter	VAP-NT Cholesterol Test	Lipoprint	Roche Cholesterol Reagent
Sample Type	Serum	Serum or plasma	Serum or plasma
Sample Size	1-2 mL	< 1mL	Instrumentation dependant
Reagent Principal	Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. The color intensity generated is directly proportional to the concentration of cholesterol and can be determined photometrically.	N/A	Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. The color intensity generated is directly proportional to the concentration of cholesterol and can be determined photometrically.
Temperature of Reaction	37C	Ambient	37C
Assay Range	32-785 mg/dl Total Cholesterol,	13-695 mg/dl LDL 5-260 mg/dl HDL	3-800 mg/dl

Parameter	VAP-NT Cholesterol Test	Lipoprint	Roche Cholesterol Reagent
	10-200 mg/dl HDL 60-250 mg/dl LDL 7-61 mg/dl for VLDL	11-140 mg/dl VLDL	
Expected Values	According to the recommendations of NCEP Adult Treatment Panel III	According to the recommendations of NCEP Adult Treatment Panel III	According to the recommendations of NCEP Adult Treatment Panel III
Risk to Patient	Provides diagnostic information, which may indirectly affect the patient such that incorrect or delayed information could result in non-serious injury to the patient	Provides diagnostic information, which may indirectly affect the patient such that incorrect or delayed information could result in non-serious injury to the patient	Provides diagnostic information, which may indirectly affect the patient such that incorrect or delayed information could result in non-serious injury to the patient
Labeling	Targeted to the professional market	Targeted to the professional market	Targeted to the professional market

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

Guidance for 510k's on Cholesterol Tests for Clinical Laboratory, Physicians' Office laboratory and Home Use

This device has not been tested by the Cholesterol Reference Method Laboratory Network

L. Test Principle:

Cholesterol is determined enzymatically using cholesterol esterase and cholesterol oxidase. The color intensity generated is directly proportional to cholesterol concentration and can be determined photometrically using spectrophotometers. The VAP-NT software interprets the photometric reaction.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Quality Control Material was the test sample material. A total of three concentrations, near medical decision points, (low, intermediate, high) were used in the precision evaluation of the VAP-NT Cholesterol Test. The table below illustrates the approximate analyte values for each of the pools.

Data Collection
Cholesterol measurements were obtained for all samples using the VAP-NT Cholesterol Test procedure. All data collection took place after device-familiarization runs.

Data was obtained over a 20 day period, using a single set of analytical equipment. Two (2) batches were run per day, and an additional 2 duplicate batches per day, for a total of 80 batches. Data for 2 replicate samples at each pool level were obtained for each batch run. One sample in a single run was excluded due to a centrifuge error. Each replicate specimen yielded analytical results for TC, HDL, LDL and VLDL, for a total of 160 data points per analyte, per level, with the exception of the high level analyte which yielded 159 data points.

The following within- run and between –run results were obtained from the analysis of the 20-day data collection period for the three levels of control material.

Total Cholesterol			Within-run		Between Run		Between Day		Total	
	n	Mean	SD	CV	SD	CV	SD	CV	SD	CV
High	160	333.1	3.59	1.1%	2.24	0.7%	1.7	0.5%	4.56	1.4%
Mid	159	227	3.96	1.7%	0	0%	0.54	0.2%	4	1.8%
Mid	160	197	2.86	1.5%	1.43	0.7%	0	0%	3.2	1.6%
Mid	160	162	2.66	1.6%	2.04	1.3%	1.64	1.0%	3.74	2.3%
Low	160	98.9	1.96	2.0%	0	0%	1.06	1.1%	2.22	2.2%

HDL			Within-run		Between Run		Between Day		Total	
	n	Mean	SD	CV	SD	CV	SD	CV	SD	CV
High	159	90.7	1.5	1.6%	0	0%	0.18	0.2%	1.51	1.7%
Mid	160	60.05	0.98	1.6%	0.43	0.7%	0.61	1.0%	1.23	2%
Mid	160	52.7	0.88	1.7%	0	0%	0	0%	0.88	1.7%
Mid	160	43.7	0.65	1.5%	0.54	1.2%	0.65	1.5%	1.06	2.4%
Low	160	34.4	0.6	1.8%	0	0%	0.36	1.0%	0.7	2.0%

LDL			Within-run		Between Run		Between Day		Total	
	n	Mean	SD	CV	SD	CV	SD	CV	SD	CV
High	160	213	2.98	1.4%	1.3	0.6%	1.79	0.8%	3.71	1.7%
Mid	159	137.5	4.03	2.9%	0	0%	1.55	1.1%	4.32	3.1%
Mid	160	117	2.65	2.3%	1.02	0.9%	0	0%	2.84	2.4%
Mid	160	93.9	2.47	2.6%	1.68	1.8%	0	0%	2.99	3.2%
Low	160	65.6	2.46	3.7%	0	0%	0	0%	2.46	3.7%

VLDL			Within-run		Between Run		Between Day		Total	
	n	Mean	SD	CV	SD	CV	SD	CV	SD	CV
High	159	52.45	2.22	4.2%	0	0%	.46	0.9%	2.27	4.3%
Mid	160	29.64	2.59	8.7%	0	0%	1.17	4.0%	2.84	9.6%
Mid	160	27.16	1.75	6.4%	0	0%	0.91	3.3%	1.97	7.2%
Mid	160	24.8	1.73	7.0%	1.09	4.4%	0%	0%	2.04	8.2%
Low	160	9.36	1.75	18.7%	0	0%	0.16	1.7%	1.76	18/8%

b. Linearity/assay reportable range:

A total of 15 serum pools were created using a high analyte starting serum pool. The starting pool (sample 4) was either serially diluted or serially concentrated to achieve a wide range of each measured analyte. The table below illustrates the theoretical pool ranges for TC.

Sample	Serum Vol (µL)	KBr Vol (µL)	Relative Concentration	Est. TC (mg/dL)
1	5	1995	0.1	26
2	20	1980	0.4	104
3	35	1965	0.7	182
4	50	1950	1.0	260
5	65	1935	1.3	338
6	80	1920	1.6	416
7	95	1905	1.9	494
8	110	1890	2.2	572
9	125	1875	2.5	650
10	140	1860	2.8	728
11	155	1845	3.1	806
12	170	1830	3.4	884
13	185	1815	3.7	962
14	200	1800	4.0	1040
15	215	1785	4.3	1118

Based on the above dilution ratios, the table below illustrates the estimated subclass values for the serial dilutions

Sample	Relative Concentration	Est. HDL	Est. LDL	Est. VLDL
1	0.1	6	16	4
2	0.4	24	64	16
3	0.7	42	112	28
4	1.0	60	160	40
5	1.3	78	208	52
6	1.6	96	256	64
7	1.9	114	304	76
8	2.2	132	352	88
9	2.5	150	400	100
10	2.8	168	448	112
11	3.1	186	496	124
12	3.4	204	544	136
13	3.7	222	592	148
14	4.0	240	640	160
15	4.3	258	688	172

Data Collection

Cholesterol measurement was obtained for all samples using the VAP-NT Cholesterol Test procedure. Data for 8 replicate samples at each pool level were obtained. A total of 12 batches were run. Two runs were rejected due to improper dilution techniques. Data collection took place within a single day, using a single set of analytical equipment. Three ultracentrifuges were used. The replicate samples were sequenced randomly for each run. Three individual samples (one each from Pool 2, 10 and 14) were eliminated for analysis due to a centrifuge error (tube collapsed). Each replicate specimen yielded analytical results for TC, HDL, LDL and VLDL, for a total of 117 data points per

analyte.

Data Analysis
The data was plotted and examined for excessive differences (errors) and potential outliers. The plot was created of the results with the observed results (Y) on the vertical axis and the theoretical sample concentration on the (X) horizontal axis.

Cholesterol y=0.9758x+7.397 r^2=0.9997 for the range of 32-785 mg/dL
HDL-C y=0.9645x+3.4159 r^2=0.9998 for the range of 10-200 mg/dL
LDL-C y=0.9867x-5.2267 r^2=0.9992 for the range of 60-250 mg/dL
VLDL-C y=1.1325x+2.89 r^2=0.9986 for the range of 7-61 mg/dL

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

The VAP-NT Cholesterol Test uses a commercially available cholesterol reagent from Roche Diagnostics. This reagent was reviewed and cleared for commercial distribution by the FDA under k952127.

d. Detection limit:

A limit of detection study was performed based upon CLSI EP-17 – Protocols for determination of Limits of Detection and Limits for Quantitation. The Limit of blank (LOB) was assessed on 60 saline/KBr Samples. In addition 60 serum samples were used to establish the Limit of detection (LOD) per CLSI EP-17. The following results were obtained.

Analyte	LOB	LOD
TC	6.1	29.5
HDL	2.6	9.4
LDL	2.6	14.1
VLDL	1.4	6.0

e. Analytical specificity:

Interferences
The VAP-NT Cholesterol Test uses a commercially available cholesterol reagent from Roche Diagnostics. This reagent was reviewed and cleared for commercial distribution by the FDA under 510(k) K952127.

As a part of the Roche reagent development, interference studies/analysis were performed and yielded the following information:

Icterus: No significant interference from conjugated bilirubin up to an I index of 25. No significant interference from unconjugated bilirubin up to an I index of 10.

Hemolysis: No significant interference from hemolysis up to an H index of 700.

Lipemia: No significant interference from lipemia up to an L index of 1250.

As the VAP-NT test uses a reagent with known limitations, the interfering substances and conditions for the Roche reagent have been incorporated into the VAP-NT Cholesterol Test labeling.

In accordance with the NCEP ATP III guidelines, the VAP-NT cholesterol test procedure will recommend patient samples be obtained from fasting donors.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The VAP-NT was compared to standard laboratory methods for the measurement of total cholesterol and cholesterol subclasses. For Total Cholesterol, the VAP-NT was compared to the Abell-Kendall method and Beta Quantification, and for the subclasses the VAP-NT was compared to Beta Quantification. The results of the study yielded to following method comparison data for the VAP-NT.

	Total Cholesterol (BQ)		**HDL**		**LDL**		**VLDL**	
	VAP	**Ref**	**VAP**	**Ref.**	**VAP**	**Ref.**	**VAP**	**Ref.**
n=	492	492	416	416	416	416	336	336
Ranges	89-404		24-90		53-188		0-60	
Slope	1.0373		0.9645		1.0037		0.951965	
Intercept	-4.3966		2.8310		0.6887		2.52943	
Coefficient of Determination (r^2)	0.9917		0.9766		0.9870		0.884145	
Average Bias (mg/dl)	6.09		2.46		3.58		See table below	
Average Bias %	2.49%		5.41%		3.33%		See table below	

VLDL Bias

Group	N	Range (mg/dL)	Average Bias mg/dL	Bias %	Bias SD
Low	112	9-17	2.69	19.07	1.9
Mid	112	18-29	3.35	13.69	2.7
High	112	30-60	3.90	10.63	3.6

b. Matrix comparison:

Not Applicable.

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:

Expected Values

Guidelines for reference ranges have been suggested by the Panel of the National Institutes of Health's Cholesterol Consensus Development Conference and adopted by the National Cholesterol Education Program. The suggested guidelines of the Panel are as follows:

Total Cholesterol Value	Risk levels Classification
< 200 mg/dL (5.17 mMol/L)	Desirable blood cholesterol
200-239 mg/dL (5.17 - 6.18 mMol/L)	Borderline-high blood cholesterol
≥ 240 mg/dL (6.20 mMol/L	High blood cholesterol

HDL Cholesterol	Classification
<1.0 mmol/L 40.00 mg/dL	Low (undesirable, High risk)
≥60.00 mg/dl	High (desirable, Low risk)

LDL Level	Classification
<100 mg/dL	Optimal
100 - 129 mg/dL	Near optimal/above optimal

130 - 159 mg/dL	Borderline High
160 - 189 mg/dL	High
≥ 190 mg/dL	Very High

VLDL Level	Classification
≤30 mg/dL	Normal
>30 mg/dL	Not Normal

N. Instrument Name:

VAP-NT Cholesterol

O. System Descriptions:

1. Modes of Operation:

   Random channel, batch mode analyzer

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:

   Bar Coded sample Identification

4. Specimen Sampling and Handling:

   The VAP-NT Test method consists of a sample preparation phase in which serum is mixed with Potassium Bromide solution to adjust density and layered beneath a saline solution, the sample is then spun using an ultra-centrifuge and vertical rotor to obtain a density gradient.

5. Calibration:

   The VAP-NT system is calibrated so that the total area under the VAP profile yields the measured total cholesterol (in mg/dL). The Calibrator material for use with the VAP-NT Cholesterol Test is bulk, filtered serum obtained from an outside research laboratory.

6. Quality Control:

   The Control material for use with the VAP-NT Cholesterol Test is bulk, filtered serum obtained from an outside research laboratory.

   Atherotech recommends that a full rotor (batch) of control samples be run and reviewed at the beginning of each shift/work period to verify analysis is within specification.

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

Not Applicable

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.