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

A. 510(k) Number:

k112538

B. Purpose for Submission:

Additional or Expanded Indications: adding lithium heparin plasma as a new claim

C. **Measurand:**

Cholesterol, High Density Lipoprotein, Low Density Lipoprotein, and Triglycerides

D. Type of Test:

Quantitative, colorimetric

E. Applicant:

Alfa Wassermann Diagnostic Technologies, LLC.

F. Proprietary and Established Names:

ACE Cholesterol Reagent
ACE HDL-C Reagent
ACE LDL-C Reagent
ACE Triglycerides Reagent

G. Regulatory Information:

Name	**Regulation**	**Product Code**	**Classification**
ACE Cholesterol Reagent	21 C.F.R. § 862.1175	CHH	I, meets limitations per 21 CFR 862.9(c)(4)
ACE HDL-C Reagent	21 C.F.R. § 862.1475	LBS	I, meets limitations per 21 CFR 862.9(c)(4)
ACE LDL-C Reagent	21 C.F.R. § 862.1475	MRR	I, meets limitations per 21 CFR 862.9(c)(4)

ACE Triglycerides Reagent	21 C.F.R. § 862.1705	CDT	I, meets limitations per 21 CFR 862.9(c)(4)

Panel: Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indications(s) for use:

   ACE Cholesterol Reagent is intended for the quantitative determination of cholesterol in serum and lithium heparin plasma using the ACE and ACE Alera Clinical Chemistry Systems. Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders. For in vitro diagnostic use only.

   ACE HDL-C Reagent is intended for the homogeneous, quantitative determination of HDL cholesterol (HDL-C) in serum and lithium heparin plasma using the ACE and ACE Alera Clinical Chemistry Systems. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases. For *in vitro* diagnostic use only.

   ACE LDL-C Reagent is intended for the quantitative determination of low density lipoprotein cholesterol (LDL-C) in serum and lithium heparin plasma using the ACE and ACE Alera Clinical Chemistry Systems. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders (such as diabetes mellitus), atherosclerosis, and various liver and renal diseases. For in vitro diagnostic use only.

   ACE Triglycerides Reagent is intended for the quantitative determination of triglycerides in serum and lithium heparin plasma using the ACE and ACE Alera Clinical Chemistry Systems. Triglyceride measurements are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism or various endocrine disorders. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   For prescription use.

4. Special instrument requirements:

ACE and ACE Alera Clinical Chemistry Systems

I. Device Description:

ACE Cholesterol Reagent
The ACE Cholesterol Reagent, used with the ACE and ACE Alera Clinical Chemistry System, is intended for quantitative *in vitro* diagnostic determination of cholesterol concentration in serum and lithium heparin plasma. It is based on a photometric test using a coupled enzymatic reaction (cholesterol esterase, cholesterol oxidase and peroxidase), which produces color via the Trinder reaction. It is composed of one reagent bottle, which is placed in the reagent compartment of the ACE or ACE Alera. It is intended for in vitro diagnostic use.

ACE HDL-C Reagent
The ACE HDL-C Reagent, used with the ACE and ACE Alera Clinical Chemistry System, is intended for quantitative *in vitro* diagnostic determination of high density lipoprotein cholesterol concentration in serum and lithium heparin plasma. It is based on a photometric test measuring the amount of HDL present utilizing two reagents, the second of which contains a unique detergent. This detergent solubilizes only the HDL lipoprotein particles, thus releasing HDL cholesterol to react with the cholesterol esterase and cholesterol oxidase, in the presence of a chromogen to produce color in the reaction cuvette. The detergent also inhibits the reaction of the cholesterol enzymes with LDL and VLDL lipoproteins and chylomicrons by adsorbing to their surfaces. It is composed of two reagent bottles, which are placed in the reagent compartment of the ACE or ACE Alera. It is intended for in vitro diagnostic use.

ACE LDL-C Reagent
The ACE LDL-C Reagent, used with the ACE and ACE Alera Clinical Chemistry System, is intended for quantitative *in vitro* diagnostic determination of low density lipoprotein cholesterol concentration in serum and lithium heparin plasma. It is based on a photometric test measuring the amount of LDL present after other non-LDL cholesterol is solubilized by a detergent and then consumed by cholesterol esterase and cholesterol oxidase in a noncolor forming reaction. LDL is then solubilized by a second detergent in the presence of a chromogenic peroxidase substrate to form a purple-red color. It is composed of two reagent bottles, which are placed in the reagent compartment of the ACE or ACE Alera. It is intended for in vitro diagnostic use.

ACE Triglycerides Reagent
The ACE Triglycerides Reagent, used with the ACE and ACE Alera Clinical Chemistry System, is intended for quantitative *in vitro* diagnostic determination of triglycerides concentration in serum and lithium heparin plasma. It is based on a photometric test using a coupled enzymatic reaction (lipase, glycerol kinase, glycerol phosphate oxidase and peroxidase), which produces color via the Trinder reaction. It is composed of one reagent bottle, which is placed in the reagent compartment of the ACE or ACE Alera. It is intended for in vitro diagnostic use.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ACE Cholesterol Reagent
   ACE HDL-C Reagent
   ACE LDL-C Reagent
   ACE Triglycerides Reagent

2. Predicate 510(k) number(s):
   ACE Cholesterol Reagent: k931786
   ACE HDL-C Reagent: k931876
   ACE LDL-C Reagent: k991733
   ACE Triglycerides Reagent: k931786

3. Comparison with predicate:

Device Comparison Table: ACE Cholesterol Reagent

	New Device	**Predicate Device**
510(k) #	**K112538**	**K931786 (ACE)**
Name	ACE Cholesterol Reagent	ACE plus ISE/Clinical Chemistry System, ACE Cholesterol Reagent
Intended Use/Indications for Use	ACE Cholesterol Reagent is intended for the quantitative determination of cholesterol using the ACE and ACE Alera Clinical Chemistry Systems. For in vitro diagnostic use only.	Same
Sample Type	Serum or lithium **heparin plasma**	Serum
Instrument Platforms	ACE and ACE Alera Clinical Chemistry Systems	ACE, ACE *Alera*® and NExCT™ Clinical Chemistry Systems
Calibration	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the calibrator; the use of GEMCAL Reference Serum is recommended.	Same
Calibration Stability	30 Days	Same
On-Board Stability	30 Days	Same

	New Device	Predicate Device
510(k) #	**K112538**	**K931786 (ACE)**
Name	ACE Cholesterol Reagent	ACE plus ISE/Clinical Chemistry System, ACE Cholesterol Reagent
Method Traceability	Abell, L.L., Levy, B.B., Brodie, B.B. and Kendall, F.E., *J. Biol. Chem.* 195, 357 (1952); Allain, C.C., Poon, L.S., Chan, C.S.G, Richmond, W. and Fu, P.C., *Clin. Chem*. 20, 470 (1974).	Same
Reactive Ingredients	4-Aminoantipyrene p-Hydroxybenzoic acid Cholesterol oxidase (Nocardia) Cholesterol esterase (porcine pancreas and Pseudomonas) Peroxidase (Horseradish)	Same
Analysis Temperature	37°C	Same
Sample Volume	3 µL	Same
Precision (mg/dL)	<u>Within run</u>: Sample A: Mean 139, SD 3.0, CV 2.2% Sample B: Mean 212, SD 2.4, CV 1.1% Sample C: Mean 290, SD 5.7, CV 2.0% <u>Total</u>: Sample A: Mean 139, SD 3.2, CV 2.3% Sample B: Mean 212, SD 3.3, CV 1.6% Sample C: Mean 290, SD 6.0, CV 2.1%	Same
Matrix Comparison	x = serum, y = lithium heparin plasma ACE: Regression Equation: y = 0.985x - 1.7 Correlation Coefficient: 0.9947 Sample Range: 40-568 ACE Alera: Regression Equation: y = 0.994x - 2.5 Correlation Coefficient: 0.9934 Sample Range: 42-577	Serum only
Detection Wavelength	505/647 nm	Same

Device Comparison Table: ACE HDL-C Reagent

	New Device	**Predicate Device**
510(k) #	**K112538**	**K931786 (ACE)**
Name	ACE HDL-C Reagent	ACE plus ISE/Clinical Chemistry System, ACE HDL-C Reagent
Intended Use/Indications for Use	ACE HDL-C Reagent is intended for the homogeneous, quantitative determination of HDL cholesterol (HDL-C) using the ACE and ACE Alera Clinical Chemistry Systems. For *in vitro* diagnostic use only.	Same
Sample Type	Serum or lithium heparin plasma	Serum
Instrument Platforms	ACE and ACE Alera Clinical Chemistry Systems	ACE and ACE *Alera*® Clinical Chemistry System
Calibration	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the calibrator; the use of GEMCAL Reference Serum is recommended.	Same
Calibration Stability	30 Days	Same
On-Board Stability	30 Days	Same
Method Traceability	Gotto, A.M., Lipoprotein metabolism and the etiology of hyperlipidemia, *Hospital Practice*, 23:Suppl. 1, 4 (1988).	Same
Reactive Ingredients	Buffer Cholesterol oxidase (E. coli) Peroxidase (Horseradish) N, N-bis(4-sulphobutyl)-m-toluidine-disodium salt Accelerator Ascorbic oxidase (Curcurbita sp.)	Same
Analysis Temperature	37°C	Same
Sample Volume	3 µL	Same
Precision (mg/dL)	Within run: Sample A: Mean 32, SD 0.7, CV 2.0% Sample B: Mean 61, SD 1.2, CV 2.0%	Same

	New Device	Predicate Device
510(k) #	**K112538**	**K931786 (ACE)**
Name	ACE HDL-C Reagent	ACE plus ISE/Clinical Chemistry System, ACE HDL-C Reagent
	Sample C: Mean 85, SD 1.5, CV 1.8% <u>Total</u>: Sample A: Mean 32, SD 1.3, CV 4.2% Sample B: Mean 61, SD 2.6, CV 4.3% Sample C: Mean 85, SD 3.3, CV 3.9%	
Comparative Analysis Regression Evaluation	Regression Equation: y = 0.954x + 1.9 Correlation Coefficient: 0.988 Sample Range: 15-120 mg/dL	Same
Expected Values	40 to 60 mg/dL	Same
Matrix Comparison	x = serum, y = lithium heparin plasma ACE: Regression Equation: y = 1.015x - 0.6 Correlation Coefficient: 0.9884 Sample Range: 6-120 ACE Alera: Regression Equation: y = 0.989x + 0.4 Correlation Coefficient: 0.9874 Sample Range: 7-123	Serum only
Detection Wavelength	592/692 nm	Same

Device Comparison Table: ACE LDL-C Reagent

	New Device	**Predicate Device**
510(k) #	**K112538**	**K931786 (ACE)**
Company	Alfa Wassermann Diagnostic Technologies, LLC	Alfa Wassermann Diagnostic Technologies, LLC
Name	ACE LDL-C Reagent	ACE plus ISE/Clinical Chemistry System, ACE LDL-C Reagent
Intended Use/ Indications for Use	ACE LDL-C Reagent is intended for the quantitative determination of low density lipoprotein cholesterol (LDL-C) using the ACE and ACE Alera Clinical Chemistry Systems. For in vitro diagnostic use only.	Same
Sample Type	Serum or lithium **heparin plasma**	Serum
Instrument Platforms	ACE and ACE Alera Clinical Chemistry Systems	ACE and the NExCT Clinical Chemistry System
Calibration	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the calibrator; the use of LDL-C Calibrator is recommended**.**	Same
Calibration Stability	30 days	Same
On-Board Stability	30 days	Same
Method Traceability	Gotto, A.M., Lipoprotein metabolism and the etiology of hyperlipidemia, *Hospital Practice*, 23: Suppl. 1, 4 (1998); Crouse, J.R. et al. Studies of low density lipoprotein molecular weight in human beings with coronary artery disease *J. Lipids Res*., 26, 566 (1985).	Same
Reactive Ingredients	Cholesterol esterase Cholesterol oxidase Peroxidase 4-Aminoantipyrine Ascorbic acid oxidase Buffer N,N-bis (4-sulfobutyl)-m-toluidine, disodium salt	Same
Analysis Temperature	37°C	Same
Sample Volume	3 µL	Same

	New Device	Predicate Device
510(k) #	**K112538**	**K931786 (ACE)**
Company	Alfa Wassermann Diagnostic Technologies, LLC	Alfa Wassermann Diagnostic Technologies, LLC
Name	ACE LDL-C Reagent	ACE plus ISE/Clinical Chemistry System, ACE LDL-C Reagent
Reaction Volume (total)	418 µL	Same
Precision (mg/dL)	Within-Run: Sample A: Mean 95, SD 2.1, CV 2.2% Sample B: Mean 146, SD 3.8, CV 2.6% Sample C: Mean 196, SD 2.8, CV 1.4% Total: Sample A: Mean 95, SD 2.5, CV 2.6% Sample B: Mean 146, SD 4.7, CV 3.2% Sample C: Mean 196, SD 6.3, CV 3.2%	Same
Comparative Analysis Regression Evaluation	Regression Equation: y = 1.111x - 15.5 Correlation Coefficient: 0.9747 Sample Range: 41-226 mg/dL	Same
Expected Values	<130 mg/dL	Same
Matrix Comparison	x = serum, y = lithium heparin plasma ACE: Regression Equation: y = 1.008x - 2.6 Correlation Coefficient: 0.9954 Sample Range: 9-460 ACE Alera: Regression Equation: y = 0.995x - 1.3 Correlation Coefficient: 0.9954 Sample Range: 9-464	Serum only
Detection Wavelength	544/692 nm	Same

Device Comparison Table: ACE Triglycerides Reagent

	New Device	**Predicate Device**
510(k) #	**K112538**	**K931786 (ACE)**
Name	ACE Triglycerides Reagent	ACE plus ISE/Clinical Chemistry System, ACE Triglycerides Reagent
Intended Use/Indications for Use	ACE Triglycerides Reagent is intended for the quantitative determination of triglycerides using the ACE and ACE Alera Clinical Chemistry Systems. For in vitro diagnostic use only.	Same
Sample Type	Serum or lithium **heparin plasma**	Serum
Instrument Platforms	ACE and ACE Alera Clinical Chemistry Systems	ACE, ACE *Alera*® and the NExCT™ Clinical Chemistry Systems
Calibration	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the calibrator; the use of GEMCAL Reference Serum is recommended.	Same
Calibration Stability:	30 Days	Same
On-Board Stability	30 Days	Same
Method Traceability	Frederickson, D.S., et al., New Engl. J. Med. 276, 32 (1976); Tietz, N.W. (Ed.), Textbook of Clinical Chemistry, W.B. Saunders Co., Philadelphia, PA (1986).	Same
Reactive Ingredients	4-Aminoantipyrine adenosine 5'-triphosphate p-Chlorophenol Glycerol phosphate oxidase (Microorganism) Lipase (Pseudomonas) Peroxidase (Horseradish) Glycerol kinase (Cellulomonas) Buffer	Same
Analysis Temperature	37°C	Same
Sample Volume	3 µL	Same
Reaction Volume (total)	243 µL	Same
Precision	Within run:	Same

	New Device	Predicate Device
510(k) #	**K112538**	**K931786 (ACE)**
Name	ACE Triglycerides Reagent	ACE plus ISE/Clinical Chemistry System, ACE Triglycerides Reagent
(mg/dL)	Sample A: Mean 73 SD 1.3, CV 1.7% Sample B: Mean 142, SD 1.6, CV 1.2% Sample C: Mean 203, SD 2.4, CV 1.2% <u>Total</u>: Sample A: Mean 73, SD 1.4, CV 1.9% Sample B: Mean 142, SD 2.6, CV 1.8% Sample C: Mean 203, SD 4.0, CV 2.0%	
Comparative Analysis Regression Evaluation	Regression Equation: y = 0.993x + 1.2 Correlation Coefficient: 0.997 Sample Range: 27-531 mg/dL	Same
Expected Values	<150 mg/dL	Same
Matrix Comparison	x = serum, y = lithium heparin plasma ACE: Regression Equation: y = 1.005x - 7.9 Correlation Coefficient: 0.9977 Sample Range: 39-887 ACE Alera: Regression Equation: y = 1.007x - 7.4 Correlation Coefficient: 0.9973 Sample Range: 38-884	Serum only
Detection Wavelength	505/692 nm	Same

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

EP9-A2 Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline- Second Edition (2002).

L. Test Principle:

ACE Cholesterol Reagent

The ACE Cholesterol Reagent is composed of a single reagent bottle.

The reagent contains:

Component	Quantity
ACE Cholesterol Reagent	
4-Aminoantipyrine (AAP)	0.5 mmol/L
p-Hydroxybenzoic acid	25 mmol/L
Cholesterol oxidase (Nocardia)	>150 U/L
Cholesterol esterase (Porcine pancreas and Pseudomonas)	>240 U/L
Peroxidase (Horseradish)	>1600 U/L
Stabilizers, Preservatives, and Fillers	

Cholesterol esters in serum are completely hydrolyzed by cholesterol esterase to free cholesterol and free fatty acids. The cholesterol liberated by the esterase, plus any endogenous free cholesterol, are both oxidized by cholesterol oxidase to yield hydrogen peroxide (H_2O_2). The hydrogen peroxide then acts to oxidatively couple p-hydroxybenzoic acid and 4-aminoantipyrine (AAP) in a reaction catalyzed by peroxidase, producing a red colored quinoneimine complex which absorbs strongly at 505 nm.

Cholesterol esters $+H_2O$ $\xrightarrow{\text{Cholesterol Esterase}}$ cholesterol + fatty acids

Cholesterol $+O_2$ $\xrightarrow{\text{Cholesterol Oxidase}}$ cholest-4-ene-3-one $+H_2O_2$

H_2O_2 + p-hydroxybenzoic acid +AAP $\xrightarrow{\text{Peroxidase}}$ quinoneimine + H_2O (red color)

The amount of chromogen formed, determined by measuring the increase in absorbance, bichromatically at 505 nm/647 nm, is directly proportional to the cholesterol concentration in the sample.

ACE HDL-C Reagent

The ACE HDL-C Reagent is composed of two reagent bottles (Buffer and Color Reagent). The Buffer (R1) contains:

Component	Quantity
ACE HDL-C Buffer (R1)	
Good's Buffer	
Cholesterol oxidase (E. coli)	<1000 U/L
Peroxidase (Horseradish)	<1300 U/L

N,N-bis(4-sulphobutyl)-m-toluidine-disodium salt (DSBmT)	<1 mM
Accelerator	<1 mM
Preservative	<0.06%
Ascorbic Oxidase (Curcubita sp.)	<3000 U/L

The Color Reagent (R2) contains:

Component	Quantity
ACE HDL-C Color Reagent (R2)	
Cholesterol esterase (Pseudomonas sp.)	<1500 U/L
4-Aminoantipyrine (4-AAP)	<1 mM
Detergent	<2%
Preservative	

The HDL-C Assay utilizes two reagents, the second containing a unique detergent. This detergent solubilizes only the HDL lipoprotein particles, thus releasing HDL cholesterol to react with the cholesterol esterase and cholesterol oxidase, in the presence of a chromogen to produce color. The detergent also inhibits the reaction of the cholesterol enzymes with LDL, VLDL and chylomicron lipoproteins by adsorbing to their surfaces. The amount of chromogen formed, determined by measuring the increase in absorbance bichromatically at 592/692 nm, is directly proportional to the HDL cholesterol concentration in the sample.

HDL, LDL, VLDL, chylomicrons → Non-Reactive LDL, VLDL, chylomicrons + DSBmT

HDL → Disrupted HDL

HDL Cholesterol → Δ^4 cholestenone + H_2O_2

H_2O_2 + DSBmT + AAP → Color Development

ACE LDL-C Reagent

The ACE LDL-C Reagent is composed of two reagent bottles (Buffer and Color Reagent).

The Buffer (R1) contains:

Component	Quantity
ACE LDL-C Buffer (R1)	
MES Buffer (pH 6.3)	
Detergent 1	<1.0%
Cholesterol esterase (Cellulomonas sp.)	<1500 U/L
Cholesterol oxidase (Pseudomonas sp.)	<1500 U/L
Peroxidase (Horseradish)	<1300 ppg U/L
4-Aminoantipyrine (4-AAP)	<0.1%
Ascorbic acid oxidase (Curcubita sp.)	<3000 U/L
Preservative	<0.1%

The Color Reagent (R2) contains:

Component	Quantity
ACE LDL-C Color Reagent (R2)	
MES Buffer (pH 6.3)	
Detergent 2	<1.0%
N,N-bis(4-sulphobutyl)-m-toluidine-disodium salt (DSBmT)	<1.0 mmol/L
Preservative	<0.1%

Detergent 1 solubilizes non-LDL lipoprotein particles (HDL, VLDL and chylomicrons) and releases cholesterol. The cholesterol is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. In a second reaction, detergent 2 solublizes the remaining LDL particles and forms peroxide, via the enzymes cholesterol esterase and cholesterol oxidase. The peroxide, in the presence of peroxidase and two peroxidase substrates, 4-aminoantipyrine and DSBmT, results in a purple-red color.

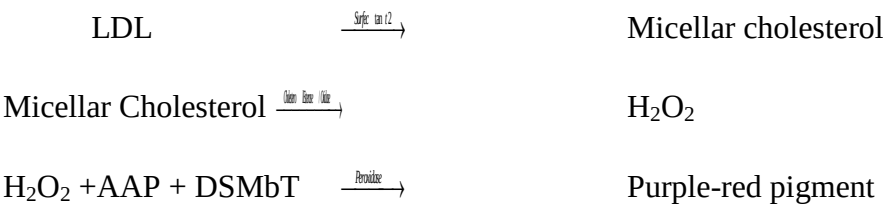
The first reaction:

HDL, VLDL $\xrightarrow{\text{Surfactant 1}}$ Micellar cholesterol

Micellar Cholesterol $\xrightarrow{\text{Cholesterol Esterase / Oxidase}}$ H_2O_2

H2O2 +AAP $\xrightarrow{\text{Peroxidase}}$ Colorless

The second reaction:



The amount of color formed, determined by measuring the increase in absorbance bichromatically at 544/692 nm, is directly proportional to the LDL cholesterol concentration in the sample.

ACE Triglycerides Reagent

The ACE Triglycerides Reagent is composed of a single reagent bottle.

The reagent contains:

Component	Quantity
ACE Triglycerides Reagent	
4-Aminoantipyrine (AAP)	0.4 mmol/L
Adenosine 5'-triphosphate (ATP)	2.6 mmol/L
p-Chlorophenol	3.0 mmol/L
Glycerol phosphate oxidase (GPO) (Microorganism)	>2400 U/L
Lipase (Pseudomonas)	>1000 U/L
Peroxidase (Horseradish)	>540 U/L
Glycerol kinase (Cellulomonas)	>400 U/L
Buffer, Stabilizers and Preservatives	

Triglycerides in serum are hydrolyzed by lipase to form glycerol and free fatty acids. In the presence of adenosine triphosphate (ATP) and glycerol kinase (GK), the glycerol is converted to glycerol-1-phosphate (G-1-P) and the ATP to adenosine diphosphate (ADP). Glycerol-1-phosphate is oxidized by glycerol phosphate oxidase (GPO) to yield hydrogen peroxide (H_2O_2). The hydrogen peroxide then acts to oxidatively couple p-chlorophenol and 4-aminoantipyrine (AAP) in a reaction catalyzed by peroxidase, producing a red colored quinoneimine complex which absorbs strongly at 505 nm.

$$\text{Triglycerides} \xrightarrow{Lipase} \text{Glycerol + fatty acids}$$
$$\text{Glycerol + ATP} \xrightarrow{GK} \text{G-1-ADP}$$
$$\text{G-1-P} + O_2 \xrightarrow{GPO} H_2O_2 + \text{dihydroxyacetone phosphate}$$
$$H_2O_2 + \text{p-chlorophenol + AAP} \xrightarrow{Peroxidase} \text{Quinomeimine (red color} + H_2O$$

The amount of chromogen formed, determined by measuring the increase in absorbance bichromatically at 505 nm/692 nm, is directly proportional to the triglycerides concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

Precision studies were performed on one lot of the ACE Cholesterol, HDL, LDL, and Triglyceride Reagents using three serum and three lithium heparin plasma samples on one ACE and one ACE Alera Clinical Chemistry Systems. The low level samples were from a normal human patient, the high levels samples were spiked with analyte, and the mid-level samples were prepared by mixing the high and low level samples. The samples were run two times per run, with two runs being performed per day, with each run consisting of each sample being run on the ACE and the ACE Alera Clinical Chemistry Systems (total n for each sample is 20). This was done for three days (HDL) or five days (Cholesterol, LDL, Triglycerides). The results are presented below.

Cholesterol on the ACE Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	145.6	2.2	1.5	3.0	2.0
Plasma 1	126.0	1.4	1.2	2.1	1.7
Serum 2	246.8	1.0	0.4	2.0	0.8
Plasma 2	332.0	4.4	1.3	5.4	1.6
Serum 3	512.2	4.9	1.0	8.9	1.7
Plasma 3	539.2	4.1	0.8	6.8	1.3

Cholesterol on the ACE Alera Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	144.5	2.0	1.4	2.8	1.9
Plasma 1	124.6	1.2	1.0	2.0	1.6
Serum 2	244.4	2.0	0.8	2.1	0.9

Plasma 2	326.4	3.1	0.9	3.9	1.2
Serum 3	503.8	3.0	0.6	5.5	1.1
Plasma 3	531.8	2.1	0.4	7.7	1.4

HDL-C on the ACE Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	51.3	1.7	3.3	1.9	3.7
Plasma 1	44.4	0.9	1.9	1.3	3.0
Serum 2	83.0	1.1	1.3	1.6	1.9
Plasma 2	76.8	0.7	0.9	0.9	1.1
Serum 3	115.4	1.0	0.8	2.1	1.8
Plasma 3	110.8	1.1	1.0	2.2	2.0

HDL-C on the ACE Alera Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	49.0	0.6	1.2	1.0	2.1
Plasma 1	42.5	0.6	1.4	1.0	2.4
Serum 2	77.9	0.8	1.0	1.2	1.6
Plasma 2	71.6	1.3	1.8	1.3	1.9
Serum 3	107.1	1.0	1.0	1.5	1.4
Plasma 3	102.6	1.3	1.2	1.9	1.9

LDL-C on the ACE Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	89.4	1.9	2.2	2.1	2.3
Plasma 1	75.4	1.7	2.3	2.3	3.0
Serum 2	154.6	2.4	1.5	3.4	2.2
Plasma 2	212.4	3.5	1.6	6.5	3.1
Serum 3	339.8	5.4	1.6	8.3	2.4
Plasma 3	360.4	6.3	1.8	6.8	1.9

LDL-C on the ACE Alera Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	89.2	2.2	2.4	2.2	2.4
Plasma 1	76.2	1.7	2.1	2.3	2.8
Serum 2	156.1	2.6	1.7	3.5	2.3
Plasma 2	214.2	3.2	1.5	4.3	2.0
Serum 3	341.4	4.3	1.3	7.8	2.3
Plasma 3	365.9	5.6	1.5	7.7	2.1

Triglyceride on the ACE Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	58.0	0.9	1.6	1.8	3.2
Plasma 1	51.0	1.2	2.4	2.0	3.9
Serum 2	329.5	2.2	0.7	4.2	1.3
Plasma 2	324.3	7.3	2.2	9.4	2.9
Serum 3	601.8	5.1	0.8	9.5	1.6
Plasma 3	596.7	4.5	0.7	7.3	1.2

Triglyceride on the ACE Alera Analyzer

Sample	Mean (mg/dL)	Within Run		Total Imprecision	
		SD	CV %	SD	CV %
Serum 1	58.1	0.6	1.1	1.7	3.0
Plasma 1	51.1	0.8	1.6	2.1	4.2
Serum 2	327.9	2.5	0.8	4.3	1.3
Plasma 2	324.6	5.4	1.7	5.4	1.7
Serum 3	599.8	4.1	0.7	4.4	0.7
Plasma 3	588.4	7.0	1.2	13.5	2.3

Precision studies were also conducted in three physician office laboratory (POL) sites on three serum samples at three concentrations over five days. The results are as follows:

Cholesterol on the ACE Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	111.4	110.5	109.5
Within-Run SD (% CV)	1.3 (1.2%)	0.8 (0.7%)	1.2 (1.1%)
Total SD (%CV)	1.4 (1.3%)	0.9 (0.8%)	1.4 (1.3%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	239.6	230.3	236.7
Within-Run SD (% CV)	2.5 (1.0%)	1.8 (0.8%)	2.1 (0.9%)
Total SD (%CV)	3.0 (1.2%)	3.0 (1.3%)	2.7 (1.1%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	364.4	347.3	356.5
Within-Run SD (% CV)	2.5 (0.7%)	3.9 (1.1%)	2.4 (0.7%)
Total SD (%CV)	4.2 (1.2%)	4.1 (1.2%)	3.1 (0.9%)

Cholesterol on the ACE Alera Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	110.5	111.3	111.0
Within-Run SD (% CV)	2.4 (2.2%)	2.4 (2.2%)	1.9 (1.7%)
Total SD (%CV)	2.6 (2.4%)	2.6 (2.4%)	2.1 (1.9%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	238.5	239.0	237.7
Within-Run SD (% CV)	4.2 (1.8%)	3.4 (1.4%)	2.5 (1.1%)
Total SD (%CV)	4.9 (2.1%)	4.4 (1.8%)	2.5 (1.1%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	356.3	359.9	358.7
Within-Run SD (% CV)	2.2 (0.6%)	6.6 (1.8%)	2.0 (0.5%)
Total SD (%CV)	4.2 (1.2%)	7.1 (2.0%)	2.9 (0.8%)

HDL on the ACE Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	35.7	33.4	35.6
Within-Run SD (% CV)	0.9 (2.4%)	0.6 (1.8%)	0.7 (2.0%)
Total SD (%CV)	1.1 (3.2%)	1.5 (4.4%)	2.1 (6.0%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	58.6	54.4	58.5
Within-Run SD (% CV)	0.9 (1.6%)	0.5 (1.0%)	1.1 (1.9%)
Total SD (%CV)	1.2 (2.1%)	2.0 (3.7%)	2.9 (5.0%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	81.2	77.3	82.0
Within-Run SD (% CV)	1.4 (1.8%)	1.0 (1.3%)	1.1 (1.3%)
Total SD (%CV)	1.8 (2.3%)	2.9 (3.8%)	4.1 (5.0%)

HDL on the ACE Alera Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	33.2	38.3	35.4
Within-Run SD (% CV)	1.1 (3.2%)	0.9 (2.3%)	0.6 (1.8%)
Total SD (%CV)	1.2 (3.7%)	1.0 (2.7%)	1.8 (5.0%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	55.0	61.2	58.0
Within-Run SD (% CV)	1.5 (2.7%)	1.2 (1.9%)	1.1 (1.9%)
Total SD (% CV)	1.9 (3.5%)	1.5 (2.5%)	2.1 (3.7%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	76.0	86.5	81.2
Within-Run SD (% CV)	1.1 (1.4%)	1.6 (1.8%)	1.5 (1.8%)
Total SD (%CV)	2.2 (2.8%)	2.2 (2.6%)	3.2 (4.0%)

___

_____

LDL on the ACE Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	67.5	67.0	67.0
Within-Run SD (% CV)	2.0 (3.0%)	0.6 (0.9%)	2.5 (3.8%)
Total SD (%CV)	2.1 (3.1%)	2.3 (3.5%)	2.7 (4.0%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	152.7	138.4	150.8
Within-Run SD (% CV)	4.1 (2.7%)	4.0 (2.9%)	3.0 (2.0%)
Total SD (%CV)	5.3 (3.5%)	4.2 (3.0%)	4.2 (2.8%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	237.1	217.8	240.2
Within-Run SD (% CV)	3.8 (1.6%)	3.3 (1.5%)	4.6 (1.9%)
Total SD (%CV)	5.2 (2.2%)	6.0 (2.8%)	6.6 (2.7%)

LDL on the ACE Alera Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	70.7	68.4	67.2
Within-Run SD (% CV)	3.3 (4.7%)	3.0 (4.4%)	1.1 (1.6%)
Total SD (%CV)	3.6 (5.0%)	3.2 (4.7%)	1.8 (2.6%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	154.8	153.6	150.8
Within-Run SD (% CV)	2.1 (1.4%)	3.6 (21.4%)	3.4 (2.3%)
Total SD (%CV)	4.5 (2.9%)	4.9 (3.2%)	3.5 (2.3%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	244.6	244.9	241.9
Within-Run SD (% CV)	3.6 (1.5%)	6.5 (2.7%)	4.3 (1.8%)
Total SD (%CV)	6.5 (2.7%)	7.1 (2.9%)	5.3 (2.2%)

Triglyceride on the ACE Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	66.7	68.8	69.3
Within-Run SD (% CV)	1.0 (1.5%)	1.5 (2.1%)	1.5 (2.1%)
Total SD (%CV)	2.0 (3.0%)	1.9 (2.8%)	1.5 (2.1%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	267.8	271.9	262.8
Within-Run SD (% CV)	1.9 (0.7%)	3.2 (1.2%)	2.1 (0.8%)
Total SD (%CV)	2.0 (0.7%)	3.7 (1.3%)	2.9 (1.1%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	450.4	461.9	439.5
Within-Run SD (% CV)	9.7 (2.1%)	8.8 (1.9%)	3.8 (0.9%)
Total SD (%CV)	11.2 (2.5%)	9.7 (2.1%)	5.1 (1.2%)

Triglyceride on the ACE Alera Analyzer

Sample 1			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	65.3	69.5	68.8
Within-Run SD (% CV)	1.9 (2.9%)	1.4 (2.1%)	1.1 (1.7%)
Total SD (%CV)	2.1 3.2%)	1.5 (2.2%)	1.8 (2.6%)
Sample 2			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	249.4	265.9	260.1
Within-Run SD (% CV)	4.8 (1.9%)	2.2 (0.8%)	1.1 (0.4%)
Total SD (%CV)	5.9 (2.4%)	2.3 (0.9%)	1.5 (0.6%)
Sample 3			
	POL 1	**POL 2**	**POL 3**
Mean (mg/dL)	410.5	442.9	432.1
Within-Run SD (% CV)	6.8 (1.7%)	3.7 (0.8%)	3.5 (0.8%)
Total SD (%CV)	7.5 (1.8%)	3.7 (0.8%)	3.7 (0.9%)

b. Linearity/assay reportable range:

The linearity remains the same as in predicate devices. The measuring ranges previously established are:
Cholesterol: 6 to 600 mg/dL
HDL: 4-125 mg/dL
LDL: 3-800 mg/dL
Triglycerides 6-1000 mg/dL

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

Not applicable, the traceability and stability remains the same as in predicate devices.

d. Detection limit:

Not applicable, the detection limit remains the same as in predicate devices.

e. Analytical specificity:
Not applicable, the specificity remains the same as in predicate devices.

f. Assay cut-off:

Not applicable.

2. Comparison studies:
   a. Method comparison with predicate device:

A method comparison study was performed using serum samples at Physician Office Laboratories. Operators assayed serum samples on the ACE and the ACE Alera Clinical Chemistry Systems. The following linear regression data was obtained by comparing samples run in house to the POL sites using Deming analysis:

ACE Analyzer

Cholesterol							
Site	**N**	**Range**	**Slope**	**95% CI of Slope**	**Intercept**	**95% CI of Intercept**	**r^2**
In-house vs. POL 1	46	41 to 431	1.017	1.001 to 1.034	-4.5	8.2 to -0.9	0.9986
In-house vs. POL 2	46	42 to 425	0.983	0.967 to 1.000	1.4	-2.4 to 5.1	0.9985
In-house vs. POL 3	46	40 to 425	0.996	0.982 to 1.009	-0.9	-3.9 to 2.0	0.9990
HDL							
In-house vs. POL 1	45	14 to 102	0.955	0.927 to 0.983	0.1	-1.4 to 1.7	0.9954
In-house vs. POL 2	45	15 to 103	0.958	0.932 to 0.984	0.8	-0.6 to 2.3	0.9960
In-house vs. POL 3	45	14 to 103	1.002	0.964 to 1.041	0.5	-1.7 to 2.6	0.9921
LDL							
In-house vs. POL 1	45	21 to 329	0.979	0.949 to 1.009	0.0	-4.2 to 4.1	0.9949
In-house vs. POL 2	45	23 to 329	0.955	0.924 to	1.7	-2.5 to 5.9	0.9945

				0.986			
In-house vs. POL 3	45	25 to 331	0.984	0.962 to 1.006	0.2	-2.8 to 3.2	0.9973
Triglyceride							
In-house vs. POL 1	47	31 to 794	1.046	1.033 to 1.060	-4.1	-7.8 to -0.4	0.9991
In-house vs. POL 2	46	28 to 789	1.023	1.002 to 1.045	-3.8	-9.7 to 2.1	0.9976
In-house vs. POL 3	46	29 to 762	1.006	0.997 to 1.016	-0.5	-3.0 to 2.1	0.9995

ACE Alera Analyzer

Cholesterol							
Site	**N**	**Range**	**Slope**	**95% CI of Slope**	**Intercept**	**95% CI of Intercept**	**r^2**
In-house vs. POL 1	46	43 to 429	1.001	0.961 to 1.042	-5.1	-14.5 to 4.2	0.9911
In-house vs. POL 2	46	43 to 428	1.002	0.971 to 1.032	-6.1	-13.2 to 1.0	0.9948
In-house vs. POL 3	46	43 to 426	0.996	0.973 to 1.019	-2.7	-8.0 to 2.6	0.9971
HDL							
In-house vs. POL 1	45	12 to 101	0.968	0.918 to 1.017	0.5	-2.3 to 3.2	0.9863
In-house vs. POL 2	45	16 to 102	0.959	0.910 to 1.008	1.6	-1.1 to 4.3	0.9862

In-house vs. POL 3	44	14 to 103	0.953	0.916 to 0.989	0.3	-1.7 to 2.4	0.9924
LDL							
In-house vs. POL 1	46	24 to 333	0.982	0.929 to 1.035	2.5	-4.9 to 9.8	0.9840
In-house vs. POL 2	46	23 to 325	0.983	0.920 to 1.045	-0.2	-8.8 to 8.4	0.9782
In-house vs. POL 3	46	20 to 322	1.011	0.984 to 1.038	-4.7	-8.3 to -1.0	0.9962
Triglyceride							
In-house vs. POL 1	46	24 to 333	0.977	0.956 to 0.999	-1.0	-6.9 to 4.9	0.9975
In-house vs. POL 2	46	31 to 755	0.997	0.970 to 1.023	0.5	-6.9 to 8.0	0.9962
In-house vs. POL 3	46	29 to 750	0.977	0.957 to 0.997	-1.0	-6.6 to 4.7	0.9977

b. Matrix comparison:

Matrix comparison was done by using Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (2002), CLSI EP9-A2 as a guideline. Paired samples were drawn from the same patients in serum and lithium heparin plasma tubes drawn at hospital sites. Some of the samples were spiked with a solution containing a high level of analyte obtained from commercial sources.

Reagent	Sample #	# Diluted/ # Spiked	Serum Range	Results ACE	Results ACE Alera
Cholesterol	ACE: 102 Alera: 103	0/5 ACE 0/6 Alera	ACE: 40 to 568 mg/dL Alera: 42 to 577 mg/dL	Slope: 0.985(0.965, 1.005) Intercept: -1.7 (-5.7 to 2.3) Correlation: 0.9947	Slope: 0.994 (0.971, 1.016) Intercept: -2.5 (-7.0, 2.1) Correlation: 0.9934
HDL	ACE: 101 Alera: 100	0/0	ACE: 6 to 120 mg/dL Alera: 7 to 123 mg/dL	Slope: 1.015 (0.984, 1.045) Intercept: -0.6 (-2.1, 0.8) Correlation: 0.9884	Slope: 0.989 (0.957, 1.02) Intercept: 0.4 (-1.2, 1.9) Correlation: 0.9874
LDL	99	0/4	ACE: 9 to 460 mg/dL Alera: 9 to 464 mg/dL	Slope: 1.008 (0.989, 1.028) Intercept: -2.6 (-5.0, -0.2) Correlation: 0.9954	Slope: 0.995 (0.976, 1.014) Intercept: -1.3 (-3.7, 1.0) Correlation: 0.9954
Triglyceride	101	0/5	ACE: 39 to 887 mg/dL Alera: 38 to 884 mg/dL	Slope: 1.005 (0.991, 1.019) Intercept: -7.9 (-11.1, -4.7) Correlation: 0.9977	Slope: 1.007 (0.992, 1.021) Intercept: -7.4 (-10.8, -4.0) Correlation: 0.9973

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

   Specific ranges for each analyte/methodology are listed in the package insert.

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.