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

A. 510(k) Number:

k113436

B. Purpose for Submission:

New device

C. Measurand:

Alkaline Phosphatase, Amylase, and Lactate Dehydrogenase

D. Type of Test:

Quantitative, enzymatic activity

E. Applicant:

Alfa Wassermann Diagnostic Technologies, LLC

F. Proprietary and Established Names:

ACE Alkaline Phosphatase Reagent
Amylase Reagent
ACE LDH-L Reagent

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CJE	II	862.1050, Alkaline phosphatase or isoenzymes test system	75-Chemistry
CIJ	II	862.1070, Amylase test system	75-Chemistry
CFJ	II, exempt, meets limitations of exemption. 21 CFR 862.9 (c) (4) and (9)	862.1440, Lactate dehydrogenase test system	75-Chemistry

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The ACE Alkaline Phosphatase Reagent is intended for the quantitative determination of alkaline phosphatase activity in serum using the ACE Axcel Clinical Chemistry System. Measurements of alkaline phosphatase are used in the diagnosis and treatment of liver, bone, parathyroid and intestinal diseases. This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.

   The ACE Amylase Reagent is intended for the quantitative determination α-amylase activity in serum using the ACE Axcel Clinical Chemistry System. Amylase measurements are used primarily for the diagnosis and treatment of pancreatitis (inflammation of the pancreas). This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.

   The ACE LDH-L Reagent is intended for the quantitative determination of lactate dehydrogenase activity in serum using the ACE Axcel Clinical Chemistry System. Lactate dehydrogenase measurements are used in the diagnosis and treatment of liver diseases such as acute viral hepatitis, cirrhosis, and metastatic carcinoma of the liver, cardiac diseases such as myocardial infarction and tumors of the lung and kidneys. This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):

   For *in vitro* diagnostic use only. For prescription and point-of-care use.

4. Special instrument requirements:

   ACE Axcel Clinical Chemistry System

I. Device Description:

The ACE Alkaline Phosphatase Reagent and ACE LDH-L Reagent for the Axcel Clinical Chemistry System each come in a kit containing 6 liquid ready-to-use bottles; three bottles containing 30 mL of R1 (reagent 1), and three bottles containing 12 mL of R2 or (reagent 2). The ACE Amylase Reagent for the Axcel Clinical Chemistry System comes in a kit containing 3 liquid ready-to-use bottles containing 30 mL of R1 (reagent 1). Buffers contain preservatives (sodium azide) and stabilizers.

J. Substantial Equivalence Information:

1. Predicate device name(s):

ACE Clinical Chemistry System, ACE Alkaline Phosphatase Reagent
ACE Clinical Chemistry System, ACE Amylase Reagent
ACE Clinical Chemistry System, ACE LDH-L Reagent

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

k931786

3. Comparison to predicate

Comparison for Alkaline Phosphatase (ALP):

	Candidate Device	**Predicate Device**
510(k) #	**k113436**	**k931786**
Similarities		
Intended Use/ Indications for Use	Same	ACE Alkaline Phosphatase Reagent is intended for the quantitative determination of alkaline phosphatase activity in serum.
Calibration	Same	Enzyme activity is directly determined by multiplying the change in absorbance per minute of the unknown samples by a constant factor based on the molar absorbtivity of p-nitrophenoxide
Method Traceability	Same	Bowers, G.N. Jr. and McComb, R.B., *Clin. Chem* 12, 70 (1966); Tietz, N.W. et al., *Clin. Chem*. 29, 751 (1983).
Use of Controls	Same	Two levels of control per day
Basic Principle	Same	Enzymatic assay for alkaline phosphatase
Measurement Type	Same	Reaction of alkaline phosphatase with colorless substrate (p-nitrophenylphosphate) in alkaline solution results in formation of p-nitrophenol and inorganic phosphate. measured spectrophotometrically at 408/486 nm
Reactive Ingredients	Same	p-Nitrophenyl phosphate, Magnesium salt, AMP buffer (pH 10.45)
Non-reactive Ingredients	Same	Preservatives and activators
Dimensions	Same	Bottles with total volumes of 12 and 30 mL of reagent
Analysis	Same	37^{o}C

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
Temperature		
Reaction Type	Same	Kinetic
Sample Type	Same	Serum
Sample Volume	Same	4 µL
Reaction Volume (total)	Same	169 µL
Differences		
Instrument Platforms	ACE Axcel Clinical Chemistry System	ACE and ACE *Alera*® Clinical Chemistry Systems
Detection Limit	1.3 U/L	2 U/L
Reportable Range	9 to 1400 U/L	2 to 1400 U/L
Endogenous Interferences	Bilirubin: For the low pool, no significant interference occurred below 27.95 mg/dL. Positive interference (19%) occurred at 55.9 mg/dL. For the high pool, no significant interference occurred. Hemolysis: For the low pool, no significant interference occurred below 62.5 mg/dL. Negative interference (≥16%) occurred at ≥125 mg/dL. For the high pool, no significant interference occurred below 500 mg/dL. A MXINIT flag occurred at 1000 mg/dL for both pools. Lipemia (Intralipid): For low and high pools, no significant interference occurred below 1000 mg/dL. A MXINIT flag occurred at 2000 mg/dL. Ascorbic Acid: No significant interference.	Bilirubin: No significant interference below 12.5 mg/dL. Concentrations greater than 12.5 may cause interference. Samples with 25 mg/dL were found to positively interfere. Hemolysis: No significant interference below 125 mg/dL. Concentrations greater than 125 may cause interference. Samples with 250 mg/dL were found to positively interfere. Lipemia (Intralipid): No significant interference.
Precision (U/L)	Within run: Sample A: Mean 50.0, SD 1.6, CV 3.2% Sample B: Mean 690.0, SD 9.8,	Within run: Sample A: Mean 42, SD 1.0, CV 2.4% Sample B: Mean 161, SD 2.5, CV 1.6% Sample C: Mean 332, SD 5.7, CV 1.7%

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
	CV 1.4% Sample C: Mean 1020.6, SD 13.2, CV 1.3% Sample D: Mean 60.9, SD 1.6, CV 2.6% <u>Total</u>: Sample A: Mean 50.0, SD 2.2, CV 4.4% Sample B: Mean 690.0, SD 19.9, CV 2.9% Sample C: Mean 1020.6, SD 28.3, CV 2.8% Sample D: Mean 60.9, SD 2.9, CV 4.7%	<u>Total</u>: Sample A: Mean 42, SD 1.5, CV 3.6% Sample B: Mean 161, SD 4.3, CV 2.7% Sample C: Mean 332, SD 9.1, CV 2.7%
Comparative Analysis Regression Evaluation	Regression Equation: y = 0.983x + 0.6 Correlation Coefficient of 0.9997 Sample Range: 12- 1363 U/L	Regression Equation: y = 0.984x -1.3 Correlation Coefficient: 0.9995 Sample Range: 14-1139 U/L
Expected Values	44-147 U/L	35-123 U/L
Sample Stability	Serum ALP is stable for 7 days at 2-8°C and for 3 months at -20°C	Serum ALP is stable for 7 days at 2-8°C and for 3 months at -20°C
Detection Wavelength	408/486 nm	408/486 nm
Reagent Stability	Unopened ACE Alkaline Phosphatase Reagent is stable until the expiration date shown on the box and bottle labels when stored in the refrigerator at 2-8°C.	Unopened ACE Alkaline Phosphatase Reagent is stable until the expiration date shown on the box and bottle labels when stored in the refrigerator at 2-8°C.
Testing Environment	Clinical laboratories or physician office laboratories	Clinical laboratories or physician office laboratories

Comparison for Amylase:

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
Similarities		
Intended Use/ Indications	Same	ACE Amylase Reagent is intended for the quantitative determination of

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
for Use		α-amylase activity in serum
Calibration	Same	Enzyme activity directly determined by multiplying the change in absorbance per minute of the unknown samples by a constant factor based on the molar absorbtivity of 2-chloro-p-nitrophenol
Method Traceability	Same	Tietz, N.W. (Ed.), *Fundamentals of Clinical Chemistry*, W.B. Saunders Co., Philadelphia, PA (1986); Sarber, R.L., Lishvin, L., Ramussen, J. and Blair, H.E., *Clin. Chem*. 32, 1136 (1986).
Use of Controls	Same	Two levels of control per day
Basic Principle	Same	Enzymatic assay for α-amylase
Measurement Type	Same	Reaction of α-amylase with chromogenic substrate (2-chloro-p-nitrophenyl-α-D-maltotrioside results in formation of 2-chloro-p-nitrophenol measured spectrophotometrically at 408/647 nm
Reactive Ingredients	Same	2-Chloro-p-nitrophenyl-α-D-maltotrioside Potassium thiocyanate Sodium chloride Calcium acetate MES buffer (pH 6.0)
Non-reactive Ingredients	Same	Preservative
Dimensions	Same	Bottles with total volumes of 12 mL of reagent
Analysis Temperature	Same	37°C
Reaction Type	Same	Kinetic
Sample Type	Same	Serum
Sample Volume	Same	3 µL
Reaction Volume	Same	168 µL

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
(total)		
Sample Stability	Same	Serum amylase is stable for 7 days at room temperature (18-26°C) and at 2-8°C for one month. Recommended storage is at 2-8°C.
Detection Wavelength	Same	408/647 nm
Testing Environment	Same	Clinical laboratories or physician office laboratories
Differences		
Instrument Platforms	ACE Axcel Clinical Chemistry System	ACE, ACE *Alera*® and NExCT™ Clinical Chemistry Systems
Detection Limit	8.5 U/L	0 U/L
Reportable Range	9 to 1900 U/L	0 to 1900 U/L
Endogenous Interferences	Bilirubin: For the low pool, no significant interference occurred below 27.95 mg/dL. Positive interference (19%) occurred at 55.9 mg/dL. For the high pool, no significant interference occurred. Hemolysis: For the low pool, no significant interference occurred below 62.5 mg/dL. Negative interference (≥21%) occurred at ≥125 mg/dL. For the high pool, no significant interference occurred. Lipemia (Intralipid): For low and high pools, no significant interference occurred below 1000 mg/dL. A MXINIT flag occurred at 2000 mg/dL. Ascorbic Acid: No significant interference.	Bilirubin: No significant interference below 16.6 mg/dL. Concentrations greater than 16.6 mg/dL may cause interference. Samples with 33.2 mg/dL were found to positively interfere. Hemolysis: No significant interference. Lipemia (Intralipid): No significant interference.
Precision (U/L)	Within run: Sample A: Mean 50.7, SD 1.7, CV 3.4% Sample B: Mean 849.2, SD 15.6, CV 1.8% Sample C: Mean 1619.6, SD 24.2, CV 1.5% Sample D: Mean 64.1, SD 1.7, CV 2.6%	Within run: Sample A: Mean 53, SD 1.3, CV 2.4% Sample B: Mean 112, SD 3.2, CV 2.9% Sample C: Mean 444, SD 10.9, CV 2.4% Total: Sample A: Mean 53, SD 2.2, CV

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
	Total: Sample A: Mean 50.7, SD 1.8, CV 3.6% Sample B: Mean 849.2, SD 16.8, CV 2.0% Sample C: Mean 1619.6, SD 26.9, CV 1.7% Sample D: Mean 64.1, SD 1.7, CV 2.7%	4.1% Sample B: Mean 112, SD 3.5, CV 3.1% Sample C: Mean 444, SD 10.5, CV 2.4%
Comparative Analysis Regression Evaluation	Regression Equation: y = 0.958x + 0.7 Correlation Coefficient of 0.9997 Sample Range: 11-1650 U/L	Regression Equation: y = 1.032x - 5.2 Correlation Coefficient: 0.9990 Sample Range: 16-1444 U/L
Expected Values	20-104 U/L	25-125 U/L

Comparison for Alkaline Phosphatase (ALP):

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
	Similarities	
Intended Use/ Indications for Use	Same	ACE LDH-L Reagent is intended for the quantitative determination of lactate dehydrogenase activity in serum.
Calibration	Same	Enzyme activity directly determined by multiplying the change in absorbance per minute of the unknown samples by a constant factor based on the molar absorptivity of NADH.
Calibration Stability	Same	Not a calibrated test
Method Traceability	Same	Wacker, W.E.C., Ulmer, D.D, Vallee, B.L., *New Engl. J. Med.*, 255, 449 (1956).
Use of Controls	Same	Two levels of control per day
Basic Principle	Same	Conversion of L-lactate to pyruvate wherein NAD is converted to NADH
Measurement Type	Same	The rate of formation of NADH product is measured bichromatically at 340/647 nm.

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
Reactive Ingredients	Same	L-lactate Nicotinamide adenine dinucleotide
Non-reactive Ingredients	Same	Buffer, preservatives, stabilizers
Analysis Temperature	Same	37°C
Reaction Type	Same	Kinetic
Sample Type	Same	Serum
Sample Volume	Same	5 µL
Reaction Volume (total)	Same	170 µL
Expected Values	Same	100-190 U/L
Sample Stability	Same	Separated from cells, lactate dehydrogenase is stable for three days at both 2-8°C and room temperature.
Detection Wavelength	Same	340/647 nm
Testing Environment	Same	Clinical laboratories or physician office laboratories
Differences		
Instrument Platforms	ACE Axcel Clinical Chemistry System	ACE and ACE *Alera*® Clinical Chemistry Systems
Detection Limit	8.3 U/L	17 U/L
Reportable Range	11 to 850 U/L	17 to 850 U/L

	Candidate Device	Predicate Device
510(k) #	**k113436**	**k931786**
Endogenous Interferences	Bilirubin: No significant interference. Hemolysis: For the low pool, significant interference occurred at all levels tested. A HI LIN flag occurred at 1000 mg/dL. For the high pool, no significant interference occurred below 31.25 mg/dL. Positive interference (≥12%) occurred at ≥62.5 mg/dL. A HI LIN flag occurred at 500 mg/dL. A DEPL flag occurred at 1000 mg/dL. Hemolysis of red cells release lactate dehydrogenase into the sample. Do not use hemolyzed samples. Triglycerides: No significant interference. Ascorbic Acid: No significant interference.	Bilirubin: No significant interference. Hemolysis: Positive interference at 31 mg/dL Lipemia (Intralipid): Positive interference at 500 mg/dL Triglycerides: No significant interference below 460 mg/dL Ascorbic Acid: No significant interference Lactic Acid: No significant interference
Precision (U/L)	Within run: Sample A: Mean 130.5, SD 3.0, CV 2.3% Sample B: Mean 437.4, SD 7.1, CV 1.6% Sample C: Mean 720.6, SD 11.8, CV 1.6% Sample D: Mean 94.7, SD 2.9, CV 3.1% Total: Sample A: Mean 130.5, SD 4.1, CV 3.2% Sample B: Mean 437.4, SD 10.2, CV 2.3% Sample C: Mean 720.6, SD 16.5, CV 2.3% Sample D: Mean 94.7, SD 4.3, CV 4.6%	Within Run: Sample A: Mean 82, SD 5.1, CV 6.2% Sample B: Mean 122, SD 5.7, CV 4.7% Sample C: Mean 282, SD 4.6, CV 1.6% Sample D: Mean 680, SD 9.7, CV 1.4% Total: Sample A: Mean 82, SD 7.0, CV 8.5% Sample B: Mean 122, SD 7.7, CV 6.3% Sample C: Mean 282, SD 10.6, CV 3.8% Sample D: Mean 680, SD 18.4, CV 2.7%
Comparative Analysis Regression Evaluation	Regression Equation: y = 1.046x + 4.9 Correlation Coefficient of 0.9986 Sample Range: 22-829 U/L	Regression Equation: y = 0.965x + 0.7 Correlation Coefficient: 0.9994 Sample Range: 20-800 U/L

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition
CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach; Approved Guideline
CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition
CLSI EP9-A2-IR: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition
CLSI EP10-A3: Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline-Third Edition
CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

The ACE Alkaline Phosphatase Reagent for the Axcel Clinical Chemistry System is an enzymatic photometric test; alkaline phosphatase in serum catalyzes the hydrolysis of the p-nitrophenyl phosphate substrate to produce inorganic phosphate and p-nitrophenol product which is measured at 408 nm. The rate of increase of absorbance is directly proportional to the amount of alkaline phosphatase activity in the serum sample.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{(ALP/Mg2+)} \text{p-nitrophenol} + H_3PO_4$$

The ACE Amylase Reagent for the Axcel Clinical Chemistry System is an enzymatic photometric test; α-amylase in serum catalyzes the reaction of the maltotrios-linked 2-chloro-p-nitrophenol phosphate substrate to produce 2-chloro-p-nitrophenol product which is measured at 408 nm. The rate of increase of absorbance is directly proportional to the amount of amylase activity in the serum sample.

$$10CNPG3 \xrightarrow{(a\text{-Amylase})} 9CNP + CNPG2 + 9G3 + \text{glucose}$$

The ACE LDH-L Reagent for the Axcel Clinical Chemistry System is an enzymatic photometric test; LDH in serum catalyzes the conversion of the L-lactate and NAD substrates to pyruvate and NADH, and the NADH product which is measured at 340 nm. The rate of increase of absorbance from the formation of NADH is directly proportional to the amount of LDH activity in the serum sample.

$$\text{L-lactate} + NAD^+ \xrightarrow{(LDH)} \text{pyruvate} + NADH + H^+$$

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

In-house precision

Precision studies were conducted by testing human serum pools at four levels. The samples were run 2 times per run, 2 runs per day, for a total of 20 days using one instrument. Results are summarized below.Alkaline Phosphatase		Sample 1	Sample 2	Sample 3	Sample 4
	Mean (U/L)	50.0	690.0	1020.6	60.9
Within Run	SD	1.6	9.8	13.2	1.6
	%CV	3.2	1.4	1.3	2.6
Between Run	SD	0.0	0.0	7.0	1.2
	%CV	0.0	0.0	0.7	2.0
Between Day	SD	1.5	17.4	24.0	2.0
	%CV	2.9	2.5	2.4	3.3
Total	SD	2.2	2.5	28.3	2.9
	%CV	4.4	2.9	2.8	4.7

Amylase		Sample 1	Sample 2	Sample 3	Sample 4
	Mean (U/L)	50.7	849.2	1619.6	64.1
Within Run	SD	1.7	15.6	24.2	1.7
	%CV	3.4	1.8	1.5	2.6
Between Run	SD	0.0	0.0	9.4	0.0
	%CV	0.0	0.0	0.6	00.0
Between Day	SD	0.6	6.3	7.1	0.4
	%CV	1.2	0.7	0.4	0.7
Total	SD	1.8	16.8	26.9	1.7
	%CV	3.6	2.0	1.7	2.7

LDH		Sample 1	Sample 2	Sample 3	Sample 4
	Mean (U/L)	130.5	437.4	720.6	94.7
Within Run	SD	3.0	7.1	11.8	2.9
	%CV	2.3	1.6	1.6	3.1
Between Run	SD	0.7	2.7	4.2	1.9
	%CV	0.6	0.6	0.6	2.0

Between Day	SD	2.7	6.7	10.9	2.6
	%CV	2.1	1.5	1.5	2.8
Total	SD	4.1	10.2	16.5	4.3
	%CV	3.2	2.3	2.3	4.6

Point-of-Care precision

Precision studies were also conducted at three Physician Office Laboratories (POL) with four trained operators typically found in these settings. Human serum pools at three concentrations were tested three times a day for five days on three instruments (one at each lab). The results are presented below:

Alkaline Phosphatase			Within Run		Total	
Lab	Sample	Mean (U/L)	SD	%CV	SD	%CV
POL 1	1	49.9	2.4	4.8	2.9	5.7
POL 2	1	49.4	1.4	2.8	1.7	3.5
POL 3	1	48.3	0.6	1.3	1.3	2.7
POL 1	2	679.5	7.1	1.0	31.8	4.7
POL 2	2	664.7	8.5	1.3	23.8	3.6
POL 3	2	676.1	7.8	1.2	27.5	4.1
POL 1	3	1340.9	20.7	1.5	43.7	3.3
POL 2	3	1293.4	15.9	1.2	56.3	4.4
POL 3	3	1041.0	12.4	1.2	21.2	2.0

Amylase			Within Run		Total	
Lab	Sample	Mean (U/L)	SD	%CV	SD	%CV
POL 1	1	53.3	2.5	4.7	2.5	4.7
POL 2	1	50.1	1.9	3.8	2.9	5.7
POL 3	1	55.0	2.3	4.2	2.3	4.2
POL 1	2	908.9	10.5	1.2	16.1	1.8
POL 2	2	872.7	30.9	3.5	41.6	4.8
POL 3	2	945.6	8.6	0.9	18.0	1.9
POL 1	3	1749.3	25.0	1.4	41.7	2.4
POL 2	3	1669.1	13.6	0.8	30.9	1.9
POL 3	3	1782.5	16.9	0.9	16.9	0.9

LDH			Within Run		Total	
Lab	Sample	Mean (U/L)	SD	%CV	SD	%CV

POL 1	1	126.1	1.8	1.4	3.1	2.5
POL 2	1	132.3	3.0	2.2	3.0	2.2
POL 3	1	130.9	2.3	1.8	2.8	2.2
POL 1	2	420.8	12.6	3.0	13.8	3.3
POL 2	2	442.1	8.3	1.9	8.3	1.9
POL 3	2	442.1	6.8	1.5	9.0	2.0
POL 1	3	701.1	9.6	1.4	12.4	1.8
POL 2	3	727.2	15.2	2.1	18.9	2.6
POL 3	3	738.3	8.4	1.1	12.3	1.7

b. Linearity/assay reportable range:

Linearity across the assay range was confirmed by spiking serum samples to a high concentration of analyte, then diluting the sample to obtain between 12 and 15 levels to cover the measuring range of each assay. The assigned value of the highest sample was set to its mean value. The assigned values of the other levels were calculated by multiplying the mean value by the dilution ratios obtained from the manufacturer. Each level was tested in replicates of four. Data was analyzed to show linear regression equations and also the 2nd and 3rd polynomial equations, and all data demonstrated that the 3 devices were linear across the claimed measuring range. Results are presented below:

Alkaline Phosphatase
Linear Regression: $y = 0.973x - 1.3$, $r^2 = 0.9976$
2nd Order: $y = 0.000036x^2 + 0.925x + 4.28$
3rd Order: $y = 0.0000001x^3 - 0.00013x^2 + 1.01x - 0.260$
Claimed measuring range: 9 – 1400 U/L

Amylase
Linear Regression: $y = 1.006x + 4.8$, $r^2 = 0.9995$
2nd Order: $y = 0.00001x^2 + 1.041x - 1.62$
3rd Order: $y = -0.0x^3 + 0.0000013x^2 + 1.03x - 0.460$
Claimed measuring range: 9 – 1900 Ul/L

LDH
Linear Regression: $y = 1.015x + 7.36$, $r^2 = 0.9980$
2nd Order: $y = 0.000112x^2 + 1.11x - 1.51$
3rd Order: $y = -0.0000002x^3 + 0.000194x^2 + 1.01x + 2.65$
Claimed measuring range: 11 – 850 U/L

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

Traceability:

Calibration of the LDH-L assay is traceable to a frozen Master Pool of verification material utilized by the reagent supplier. Each lot of reagent is tested by running the Master Pool and verifying that results of the Master Pool levels are within an acceptable percentage of the assigned values of the Master Pool. For value assignment, each new verification Master Pool is made by gravimetrically adding quantities of lactate dehydrogenase to a serum pool to target concentrations. Five levels of Master Pool are prepared, aliquoted and stored at ≤-70° C. The final values of the Master Pool are assigned for each level by testing at least 3 replicates on multiple instruments. The activity levels of the new Master Pool are verified using a previously approved Master Pool lot as a control.

The ACE Alkaline Phosphatase and Amylase reagents are traceable to an IFCC traceable method, using a linearity verification set with various levels run in triplicate and assessed for linearity versus the assigned values from the linearity set.

d. Detection limit:

The limit of detection and the limit of the blank were determined by assaying five low samples (serum samples) and five true blanks (human serum albumin in saline). Testing was carried out over three days on two ACE Axcel Clinical Chemistry Analyzers. Serum samples and true blanks were assayed every day for a total of 60 measurements. The limit of quantitation was determined with 40 replicates of 3 low samples, and was determined to be the mean when the %CV was ≤ 20%.

Analyte	LoB (U/L)	LoD (U/L)	LoQ (U/L)
Alkaline Phosphatase	1.1	1.3	6
Amylase	7.9	8.5	9
LDH	7.8	8.3	11

e. Analytical specificity:

Interference studies were performed to determine the effects from potential interferents. The various concentrations of interferent were spiked into serum pools containing alkaline phosphatase, amylase and LDH at normal and abnormal concentrations. Hemolysis was simulated using a freeze-thaw method to lyse the red cells. All samples were tested in triplicate. Seven levels were tested for each interferent. Significant interference was defined as a difference in analyte recovery of more than ± 10%.

Alkaline Phosphatase:

Interferent Compound	Concentration with No Interference Up To
Ascorbic Acid	6 mg/dL

Unconjugated Bilirubin	28 mg/dL
Hemolysis (hemoglobin)	62.5 mg/dL*
Intralipid	500 mg/dL

Amylase:

Interferent Compound	Concentration with No Interference Up To
Ascorbic Acid	6 mg/dL
Unconjugated Bilirubin	28 mg/dL
Hemolysis (hemoglobin)	62.5 mg/dL*
Intralipid	1000 mg/dL

LDH:

Interferent Compound	Concentration with No Interference Up To
Ascorbic Acid	6 mg/dL
Unconjugated Bilirubin	62 mg/dL
Hemolysis (hemoglobin)	Interference at All Levels*
Triglycerides**	2620 mg/dL

*The package insert contains the following statement: Do not use hemolyzed samples.

**Triglycerides were used in the study with LDH as the sponsor suspected matrix interferences from intralipid with this analyte.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

An in-house method comparison study to the predicate device was performed with serum patient samples. A total of 113 alkaline phosphatase (103 native, 5 diluted and 5 spiked) serum samples, 111 amylase (101 native, 4 diluted and 6 spiked) serum sample, and 121 LDH (109 native, 5 dilutes and 7 spiked) serum samples covering the assay range were tested. The results are presented in the table below:

Analyte	n	Regression Equation	r^2	Standard Error	Sample range (U/L)
Alkaline Phosphatase	112	y=0.983x+0.6	0.9997	5.1	12-1363

Amylase	111	y=0.958x+0.7	0.9997	6.5	11-1650
LDH	121	y=1.046x+4.9	0.9986	7.5	22-829

Additional method comparison studies were performed at three Physician Office Laboratories, with four operators. Operators assayed serum samples ranging from 11-1388 U/L alkaline phosphatase, 12-1856 U/L amylase, and 18-819 LDH on the Ace Axcel clinical chemistry analyzer and the ACE clinical chemistry System. The results are presented in the tables below:

Alkaline Phosphatase

POL	n	Regression Equation	r^2	Standard Error	Sample range (U/L)
1	68	y=1.040x+3.5	0.9957	25.3	11-1311
2	53	y=0.972x+1.5	0.9998	6.0	49-1261
3	49	y=1.000x+8.7	0.9983	16.8	26-1388

Amylase

POL	n	Regression Equation	r^2	Standard Error	Sample range (U/L)
1	56	y=0.997x-2.5	0.9998	7.6	12-1819
2	49	y=0.984x-0.5	0.9985	22.3	19-1856
3	47	y=1.019x-1.5	1.0000	3.4	18-1797

LDH

POL	n	Regression Equation	r^2	Standard Error	Sample range (U/L)
1	60	y=1.010x-1.1	0.9983	13.1	33-819
2	53	y=1.042x-5.7	0.9993	6.3	18-773
3	47	y=1.011x+2.6	0.9988	7.9	22-787

b. Matrix comparison:

The device is being cleared for serum use only.

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. Clinical studies are not typically submitted for this device type.

5. Expected values/Reference range:

The reference range for each analyte was verified according to CLSI C28-A3. 50 normal healthy patient samples from a diverse population with an age range from 20 to 60, were each analyzed for ALP, Amylase, and LDH. The 95% confidence intervals were calculated and the reference range was shown to validate the reference range stated in the literature (for amylase and LDH: Tietz Clinical Guide to Laboratory Tests, 4th Ed, Wu *et al*; for ALP: Medline Plus reference range data base, U.S. National Library of Medicine, National Institutes of Health).

ALP: 44 – 147 U/L

Amylase: 20 – 104 U/L

LDH: 100 – 190 U/L

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.