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

A. 510(k) Number:

k131975

B. Purpose for Submission:

New reagents (Total Iron-binding capacity, Iron, Lactate dehydrogenase) added onto ACE Alera instrument (k123018).

C. Measurand:

Total Iron-binding capacity
Iron
Lactate dehydrogenase

D. Type of Test:

Quantitative, photometric methods, enzymatic activity

E. Applicant:

Alfa Wassermann Diagnostic Technologies, Inc.

F. Proprietary and Established Names:

ACE Direct Total Iron-Binding Capacity (TIBC) Reagent
ACE Total Iron Reagent
ACE LDH-L Reagent

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
JMO	Class I, reserved	21 C.F.R. §862.1415 Iron-binding capacity test system	Clinical Chemistry (75)
JIY	Class I, reserved	21 C.F.R. §862.1410 Iron (non-heme) test system	Clinical Chemistry (75)

CFJ	Class II, exempt, meets limitations of exemption. 21 CFR 862.9 (c)(9)	21 C.F.R.§ 862.1440 Lactate dehydrogenase test system	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The ACE Direct Total Iron-Binding Capacity (TIBC) Reagent is intended for the quantitative determination of total iron-binding capacity in serum using the ACE Alera Clinical Chemistry System. Iron-binding capacity measurements are used in the diagnosis and treatment of anemia. This test is intended for use in clinical laboratories and physician office laboratories. For in vitro diagnostic use only.

   The ACE Total Iron Reagent is intended for the quantitative determination of iron in serum using the ACE Alera Clinical Chemistry System. Iron (non-heme) measurements are used in the diagnosis and treatment of diseases such as iron deficiency anemia, hemochromatosis (a disease associated with widespread deposit in the tissues of two iron-containing pigments, hemosiderin and hemofuscin, and characterized by pigmentation of the skin), and chronic renal disease. This test is intended for use in clinical laboratories and 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 Alera 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 or kidneys. This test is intended for use in clinical laboratories and physician office laboratories. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   For prescription and point-of-care use.

4. Special instrument requirements:

   ACE Alera Clinical Chemistry System

I. Device Description:

ACE Direct Total Iron-Binding capacity (TIBC) Reagent assay consists of the Direct TIBC Color Reagent (R1) and the Direct TIBC Buffer (R2). The Direct TIBC Color Reagent contains chromazurol B, cetrimide, ferric chloride and acetate buffer. The Direct TIBC Buffer contains sodium bicarbonate buffer. Both reagents are added to the serum sample. Calibrators are package separately and have been previously cleared in k052148.

ACE Serum Iron Reagent is composed of two reagent bottles, Buffer (R1) and Color Reagent (R2). The Buffer contains hydroxylamine hydrochloride, acetate buffer (pH 4.5) and surfactant. The Color Reagent contains ferrozine and hydroxylamine hydrochloride. Calibrators are provided with the iron reagents (Iron standard) and have been previously cleared in k944911.

ACE LDH-L Reagent is composed of two reagent bottles (Substrate and Coenzyme Reagent). The reagents contain L-lactic acid (112 mmol/L) and nicotinamide adenine dinucleotide (11.8 mmol/L).

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) numbers:

   ACE Direct Total Iron-Binding Capacity (TIBC) Reagent: k930104
   ACE Iron Reagent: k944911
   ACE LDH-L Reagent: k931786

2. Comparison with predicate:

Similarities and Differences		
Item	Candidate Device	Predicate Device (k930104; ACE Direct Total Iron-Binding capacity (TIBC) Reagent)
Intended Use	For the quantitative determination of total iron-binding capacity in human serum.	Same
Method	Photometric	Same
Sample Type	Serum	Same
Expected value	250-425 µg/dL	250-450 µg/dL
Measuring range	52-700 µg/dL	Same
Instrument platform	ACE Alera Clinical Chemistry System	ACE Clinical Chemistry System

Similarities and Differences		
Item	Candidate device	Predicate device (k944911; ACE Iron Reagent)
Intended Use	For the quantitative determination of iron in human serum.	Same
Method	Photometric	Same
Sample Type	Serum	Same
Expected value	Male: 65-175 µg/dL Female: 50-170 µg/dL	Same
Measuring range	12-600 µg/dL	2-600 µg/dL
Instrument platform	ACE Alera Clinical Chemistry System	ACE Clinical Chemistry System

Similarities and Differences		
Item	Candidate device	Predicate device (k931786: ACE LDH-L Reagent)
Intended Use	For the quantitative determination of lactate dehydrogenase activity in human serum.	Same
Method	Photometric	Same
Sample Type	Serum	Same
Expected value	100-190 U/L	Same
Measuring range	18-850 U/L	17-850 U/L
Instrument platform	ACE Alera Clinical Chemistry System	ACE Clinical Chemistry System

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

CLSI Guideline EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods: Approved Guideline - Second Edition (2004).

CLSI Guideline EP06-A: Evaluation of the Linearity of Qualitative Measurement Procedures: A Statistical Approach (2003)

CLSI Guideline EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition (2005)

CLSI Guideline EP09-A2-IR: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (2002)

CLSI Guideline EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation (2004)

L. Test Principle:

ACEDirectTotalIron-Bindingcapacity(TIBC)Reagent
The Direct TIBC Color reagent releases iron from transferrin, forming a color complex with the dye. Then the Direct TIBC Buffer is added, shifting the pH and resulting in a large affinity of transferrin for iron. The serum transferrin rapidly binds the iron by abstracting the iron from the dye-iron complex. The decrease in absorbance is directly proportional to the total iron binding capacity of the serum sample. The absorbance is measured at 647 nm.

ACESerumIronReagent
The transferring-bound iron in serum is released at a pH and reduced from ferric to ferrous iron. These ions react with ferrozine to form a violet color complex, which is measured bichromatically at 554 nm/692 nm. The intensity of color produced is directly proportional to the serum iron concentration.

ACE LDH-L Reagent
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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were conducted in house following CLSI guidance document EP05-A2. At least 3 serum-based samples of low, mid and high analyte were run on the ACE Alera Clinical Chemistry System in duplicate, for 2 runs per day, for a minimum of 19 days (N=76 per concentration level). Results are summarized below:

ACE Alera		Precision (SD, %CV)		
		Mean	Within-Run	Total
TIBC µg/dL	Low	217	4.1, 1.9%	6.7, 3.1%
	Mid	270	3.7, 1.4%	7.1, 2.6%
	High	310	5.0, 1.6%	8.6, 2.8%
	Low	62	3.2, 5.2%	4.6, 7.3%

Iron µg/dL	Mid	145	2.2, 1.5%	4.2, 2.9%
	High	226	4.1, 1.8%	5.0, 2.2%
LDH-L U/L	1	77	3.8, 4.9%	4.2, 5.5%
	2	119	5.1, 4.3%	5.2, 4.3%
	3	270	4.5, 1.7%	5.8, 2.1%
	4	651	12.6, 1.9%	13.5, 2.1%

Precision studies were conducted at 3 Physician Office Laboratories (POL) with trained operators typically found in these settings, following CLSI guidance document EP05-A2. Three serum samples each of low, mid and high analyte were analyzed on the ACE Alera Clinical Chemistry System in duplicate, for 5 days at 2 runs per day (N=20 per analyte level at each POL site). Results are summarized below.

TIBC:

		ACE *Alera* Result		
n=20		µg/dL	SD, %CV	
Lab	Sample	Mean	Within-Run	Total
POL 1	1	284	8.3	9.6
			2.9%	3.4%
POL 2	1	259	5.6	8.5
			2.2%	3.3%
POL 3	1	276	9.1	16.7
			3.3%	6.0%
POL 1	2	464	6.3	6.6
			1.4%	1.4%
POL 2	2	444	4.2	5.4
			1.0%	1.2%
POL 3	2	453	3.2	15.5
			0.7%	3.4%
POL 1	3	544	8.2	8.3
			1.5%	1.5%
POL 2	3	520	5.0	9.0
			1.0%	1.7%
POL 3	3	533	12.6	20.2
			2.4%	3.8%

Iron:

		ACE *Alera* Result		
n=20		µg/dL	SD, %CV	
Lab	Sample	Mean	Within-Run	Total
POL 1	1	119	2.7	3.2
			2.3%	2.7%
POL 2	1	122	3.1	3.1
			2.6%	2.6%
POL 3	1	116	3.2	3.4
			2.8%	3.0%
POL 1	2	229	2.0	2.5
			0.9%	1.1%
POL 2	2	235	2.3	2.4
			1.0%	1.0%
POL 3	2	229	3.4	3.9
			1.5%	1.7%
POL 1	3	424	4.0	4.6
			0.9%	1.1%
POL 2	3	435	2.4	5.3
			0.5%	1.2%
POL 3	3	428	11.1	11.1
			2.6%	2.6%

LDH:

		ACE *Alera* Result		
n=20		µg/dL	SD, %CV	
Lab	Sample	Mean	Within-Run	Total
POL 1	1	116	1.7	4.9
			1.5%	4.3%
POL 2	1	118	3.0	5.1
			2.5%	4.3%
POL 3	1	124	3.4	4.7
			2.7%	3.8%
POL 1	2	437	2.9	5.8
			0.7%	1.3%
POL 2	2	449	3.7	5.2

			0.8%	1.2%
POL 3	2	446	5.8	6.6
			1.3%	1.5%
POL 1	3	698	8.6	11.5
			1.2%	1.6%
POL 2	3	726	5.4	10.0
			0.8%	1.4%
POL 3	3	716	14.3	16.9
			2.0%	2.4%

b. Linearity/assay reportable range:

A linearity study was conducted following CLSI guidance document EP06-A. Serum samples were spiked with the appropriate analyte and a 10 sample dilution series was created by diluting with a low level analyte sample. The assigned values of the highest and lowest sample were set to their mean values. Each level was tested in triplicate on the ACE Alera Clinical Chemistry System. The linear regression correlation between the expected values and the measured values for each of the assays is summarized below:

	Range Tested	Linear Regression fit	Correlation Coefficient
TIBC	34 - 740 µg/dL	y = 1.020x + 3.1	0.9981
Iron	6 - 666 µg/dL	y = 1.030x + 1.9	0.9986
LDH-L	8 - 895 U/L	y = 1.050x – 0.7	0.9981

The linearity data provided by the sponsor support the following reportable range claims:

Analyte	Assay range
TIBC	52-700 µg/dL
Iron	12-600 µg/dL
LDH-L	18-850 U/L

Automatic dilution study: The ACE Alera Clinical Chemistry System can perform automatic dilutions of samples that exceed the upper limit of the ACE Total Iron and ACE LDH-L assays (1:2 and 1:4 dilutions, respectively). To examine the accuracy of the auto-dilution function for these reagents, serum samples were spiked with high concentrations of analyte and were then either run directly on the ACE Alera system or they were manually diluted using system diluent (3 samples per instrument system, run in quintuplicate). The results from the auto-dilution and the manually diluted samples, run on the same ACE Alera system, were compared. All samples recovered within 10%

recovery. The auto-dilution function is not available for ACE Direct TIBC Reagent.

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

Traceability:

ACE LDH reagent

No calibrator is needed. 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.

ACE Direct Total Iron-Binding capacity (TIBC) Reagent

Traceable to another commercially available FDA cleared assay by method comparison. The calibrator was previously cleared under k052148.

ACE Serum Iron Reagent

Traceable to NIST SRM 937. The calibrator was previously cleared under k944911.

d. *Detection limit:*

Limit of Blank (LoB) and Limit of Detection (LoD) studies were performed and were determined to be adequate. Limit of Quantitation (LoQ) was determined according to CLSI guidance document EP17-A, by evaluating five low level samples with eight replicates per day over five days, for a total of 40 measurements per sample. The sponsor defined LoQ as concentration with a %CV of < 20%. The results are as follows:

ACE Alera	TIBC	Iron	LDH-L
LoB	11 µg/dL	0 µg/dL	11 U/L
LoD	24 µg/dL	1 µg/dL	18 U/L
LoQ	52 µg/dL	9.15 µg/dL	18 U/L

The detection limit studies support the sponsors claimed measuring ranges for the ACE TIBC, ACE Iron and ACE LDH-L assays.

Claimed measuring range:

Analyte	Assay range
TIBC	52-700 µg/dL
Iron	12-600 µg/dL
LDH-L	18-850 U/L

e. Analytical specificity:

Interference studies were performed, according to CLSI guidance document EP07-A2, to determine the effects of potential interferents. Various concentrations of interferents were spiked into serum pools containing analytes at normal and abnormal concentrations. All samples were tested in triplicate on the ACE Alera Clinical Chemistry system. Six interferent levels and the control samples were tested for each interferent. Bias greater than +/- 10% defines significant interference by the sponsor. The results of the highest concentration tested without significant interference are as follows:

	No Significant Interference at or below:		
	TIBC (250 and 550 µg/dL)	Iron (95 and 310 µg/dL)	LDH-L (150 and 530 U/L)
Bilirubin	59 mg/dL	59 mg/dL	50 mg/dL
Hemolysis	188 mg/dL	125 mg/dL	< 31 mg/dL
Ascorbic Acid	3 mg/dL	6 mg/dL	6 mg/dL

Intralipid concentration tested up to 1000 mg/dL did not interfere with the TIBC or LDH assays, however, intralipid concentration above 125 mg/dL will interfere with the iron assay, therefore, sponsor put a limitation in the labeling that states “Visibly lipemic samples will interfere with the Iron assay, therefore, lipemic samples should not be used.” In addition, since hemolysis

interferes with all three assays, the package inserts for the ACE TIBC, ACE Total Iron and ACE LDH-L reagents contain the following statement: "Do not use hemolyzed samples."

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 patient serum samples, in accordance with CLSI Guidance Document EP09-A2. Serum samples covering the assay range were tested in singlicate: 50 serum samples for TIBC (45 native, 3 diluted and 2 spiked), 48 serum samples for Iron (43 native, 0 diluted and 5 spiked), and 58 serum samples for LDH-L (46 native, 6 diluted and 6 spiked). Results of the candidate device were compared to the predicate device. The linear regression results are presented in the table below:

ACE results (x) vs. ACE Alera results (y):

	TIBC	Iron	LDH-L
n	50	48	58
Range tested	59 to 676 µg/dL	13 to 549 µg/dL	20 to 799 U/L
Slope	0.987	0.993	0.997
Intercept	3.6	0.9	-3.6
Corr. Coef., r^2	0.9960	0.9995	0.9982

Method comparison studies were completed at 3 POC sites following CLSI document EP09-A2. At least 48 determinations were made in singlicate for serum samples, having analyte levels covering each assay's dynamic range, at the each of 3 POC sites on the ACE Alera Clinical Chemistry System (y) and on the ACE Clinical Chemistry System (x) in-house, with the following linear regression data:

ACE Alera Clinical Chemistry System (y)		In-House (x) vs. POL 1 (y)	In-House (x) vs. POL 2 (y)	In-House (x) vs. POL 3 (y)
TIBC	n Range Regression Correlation	50 59 to 676 y = 0.994x + 12.4 0.9934	50 59 to 676 y = 0.973x + 0.1 0.9954	50 59 to 676 y = 1.005x + 9.0 0.9898
Iron	n	48	48	48

	Range Regression Correlation	13 to 549 y = 0.976x + 1.0 0.9986	13 to 549 y = 0.976x + 2.3 0.9981	13 to 549 y = 0.951x + 0.8 0.9966
LDH-L	n Range Regression Correlation	51 74 to 799 y = 0.992x + 3.5 0.9986	51 74 to 799 y = 1.027x + 3.4 0.9989	51 74 to 799 y = 1.010x + 2.5 0.9984

b. Matrix comparison:

None, the device is being cleared for serum use only.

4. 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 following expected values are provided in the product insert based on the literature for each analyte*. The sponsor stated that each laboratory should determine the expected values for its particular population.

TIBC expected values are 250-425 µg/dL.

Total Iron expected values are the following for males and females, respectively: 65-175 µg/dL and 50-170 µg/dL.

LDH-L expected values are 100-190 U/L

* Wu, A.H.B., ed. Tietz clinical guide to laboratory tests, 4th edition, p. 880. W.B. Saunders Company, St. Louis (2006).

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.