

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

**A. 510(k) Number:**

k091544

**B. Purpose for Submission:**

New device

**C. Measurand:**

Lactate Dehydrogenase

**D. Type of Test:**

Quantitative

**E. Applicant:**

Alfa Wassermann Diagnostic Technology, Inc.

**F. Proprietary and Established Names:**

S-Test Lactate Dehydrogenase (LD), Model RC 0017

**G. Regulatory Information:**

1. Regulation section:  
21 CFR § 862.1440, Lactate dehydrogenase test system
2. Classification:  
Class II
3. Product code:  
CFJ, NAD reduction/NADH oxidation, lactate dehydrogenase
4. Panel:  
Clinical Chemistry (75)

**H. Intended Use:**

1. Intended use(s):  
The S-Test Lactate Dehydrogenase Reagent is intended for the quantitative determination of lactate dehydrogenase activity in serum using the S40 Clinical Analyzer. 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 or physician office laboratories. For *in vitro* diagnostic use only.
2. Indication(s) for use:  
See Intended use(s).

3. Special conditions for use statement(s):  
For prescription use only; For *in vitro* diagnostic use
4. Special instrument requirements:  
For use with the S40 Clinical Analyzer

**I. Device Description:**

The single use cartridges are plastic containers consisting of two liquid stable reagents and a reaction cavity, together with a barcode label. The barcode contains all chemistry parameters, calibration factors, and other production-related information.

Reagent 1 contains: Lithium L-lactate (92 mmol/L) and diethanolamine buffer (pH 8.8, 460 mmol/L).

Reagent 2 contains: Nicotinamide adenine dinucleotide (oxidized type, 18 mmol/L).

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
Alfa Wassermann ACE plus/ISE Clinical Chemistry System
2. Predicate K number(s):  
k931786
3. Comparison with predicate:

Item	S-Test LD Reagent on the S40 Clinical Analyzer	ACE plus/ ISE Clinical Chemistry System (k931786)
<b>Similarities</b>		
Intended Use	The S-Test LD Reagent is intended for the quantitative determination of LD activity in serum using the S40 Clinical Analyzer. LD 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 or physician office laboratories. For <i>in vitro</i> diagnostic use only.	ACE LDH-L Reagent is intended for the quantitative determination of LD activity in serum using the ACE, ACE Alera and the NExCT clinical chemistry systems.
Analyte	LD activity	same
Basic Principle	Conversion of L-lactate to pyruvate wherein NAD is converted to NADH.	same
Analysis Temperature	37 °C	same
Reaction Type	Kinetic	same

Sample Type	Serum	same
Reagent Stability	Reagents are stable until the expiration date on the box labels when stored in the refrigerator at 2-8 °C.	same
Testing Environment	Clinical laboratories or physician office laboratories.	same
<b>Differences</b>		
Instrument Platforms	S40 Clinical Analyzer	ACE, ACE Alera and the NExCT Clinical Chemistry Systems
Calibration	Each lot calibrated by manufacturer prior to shipment using material traceable to the Japanese Committee for Clinical Laboratory Standardization approved standard Japan/Conventional Enzyme Standard Substance; 2-D barcode printed on each cartridge provides analyzer with lot-specific calibration data.	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.
Measurement Type	The rate of formation of NADH product is measured bichromatically at 340/405 nm.	The rate of formation of NADH product is measured bichromatically at 340/647 nm.
Reactive Ingredients	Lithium L-lactate, Diethanolamine buffer, Nicotinamide adenine dinucleotide (oxidized type)	L-lactate, AMP buffer, Nicotinamide adenine dinucleotide (NAD)
Sample Volume	8 µL	4 µL
Reaction Volume (total)	308 µL	169 µL
Detection Wavelength	340/405 nm	340/647 nm
Linearity Range	10 to 672 U/L	13 to 850 U/L
Detection Limit	9 U/L	13 U/L
Endogenous Interferences	Bilirubin; No significant interference Hemolysis: Positive interference ( $\geq 20\%$ ) at all levels tested. Lipemia (Intralipid): No significant interference below 750 mg/dL. Interference occurred (absorbance exceeds reaction limit) at 1000 mg/dL.	Bilirubin; No significant interference Hemolysis: Positive interference at 6 mg/dL. Lipemia (Intralipid): No significant interference below 1000 mg/dL.
Precision (U/L)	Within run: Sample A: Mean 74, SD 1.5, CV 2.0% Sample B: Mean 122, SD 2.9, CV 2.4% Sample C: Mean 280, SD 4.2, CV 1.5%  Between run: Sample A: Mean 74, SD 4.1, CV 5.6% Sample B: Mean 122, SD 7.0, CV 5.7% Sample C: Mean 280, SD 16.1, CV 5.8%	Within run: Sample A: Mean 88, SD 2.1, CV 2.4% Sample B: Mean 128, SD 6.6, CV 5.1% Sample C: Mean 294, SD 6.0, CV 2.1%  Total:

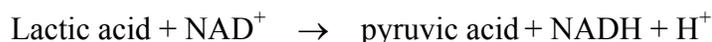
	<p>Between day:  Sample A: Mean 74, SD 2.9, CV 4.0%  Sample B: Mean 122, SD 1.8, CV 1.5%  Sample C: Mean 280, SD 8.8, CV 3.1%</p> <p>Total:  Sample A: Mean 74, SD 5.3, CV 7.1%  Sample B: Mean 122, SD 7.8, CV 6.4%  Sample C: Mean 280, SD 18.8, CV 6.7%</p>	<p>Sample A: Mean 88, SD 3.4, CV 3.8%  Sample B: Mean 128, SD 7.7, CV 6.0%  Sample C: Mean 294, SD 7.5, CV 2.6%</p>
Expected Values	<p>100 – 190 U/L</p> <p>Range was confirmed by testing 56 normal patient, ages 18-92 on the S-Test LD assay, with the following results:  Mean = 128 U/L (range 88 – 181 U/L)</p>	<p>0 - 4 days: 290 – 775 U/L  4 - 10 days: 545 – 2000 U/L  10 days – 24 months: 180 - 430 U/L  24 months – 12 years: 110 - 295 U/L  12 – 60 years: 100 - 190 U/L  60 – 90 years: 110 - 210 U/L  &gt;90 years: 99 - 284 U/L</p>

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

- Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline (EP5-A2)
- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (EP6-A)
- Interference Testing in Clinical Chemistry; Approved Guideline (EP7-A2), Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP9-A2)
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (EP17-A)
- Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline (C28-A3)

**L. Test Principle:**

LD in the sample catalyzes the conversion of L-lactate to pyruvate. Nicotinamide adenine dinucleotide (NAD<sup>+</sup>) acts as an acceptor for the hydrogen ions released from the L-lactate substrate and is converted to reduced nicotinamide adenine dinucleotide (NADH).



The rate of increase in absorbance, monitored bichromatically at 340/405 nm, is directly proportional to the LD activity in the sample.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision of the LD assay on the S40 Clinical Analyzer was evaluated using a method based on CLSI EP5-A2 at an external site. Three commercial control samples with normal, intermediate and elevated levels of LD activity were assayed on two S40 Clinical Analyzers two times per run, two runs per day, for a total of 22 days. All three samples were tested on both analyzers. Approximately 50% of the data was collected on each of the analyzers. The mean, standard deviations (SD) and % coefficient of variation (CV) were calculated for each sample. The results are summarized below:

Sample	Mean (U/L LD)	Within run		Between run		Between day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	74	1.5	2.0	4.1	5.6	2.9	4.0	5.3	7.1
2	122	2.9	2.4	7.0	5.7	1.8	1.5	7.8	6.4
3	280	4.2	1.5	16.1	5.8	8.8	3.1	18.8	6.7

To establish the precision of the LD assay on the S40 Clinical Analyzer using physical office lab (POL) personnel, three serum samples with normal, intermediate and elevated levels of LD activity were assayed on four S40 Clinical Analyzers (one at each of three POLs and one in-house) three times per run, one run per day, for a total of five days. The low sample was an unaltered human serum pool. For the two higher levels, the serum samples were spiked with LD from a commercial source. The mean, SD and % CV were calculated for each sample. The results are summarized below:

Lab	Sample	Mean (U/L LD)	Within run		Total	
			SD	CV%	SD	CV%
In-house	1	67	2.7	4.0	3.4	5.1
POL 1	1	69	2.9	4.3	2.9	4.3
POL 2	1	69	1.4	2.0	1.4	2.0
POL 3	1	70	1.0	1.4	1.0	1.4
In-house	2	194	7.9	4.1	7.9	4.1
POL 1	2	198	3.1	1.6	3.1	1.6
POL 2	2	200	2.4	1.2	2.4	1.2
POL 3	2	200	2.5	1.3	2.6	1.3
In-house	3	519	11.2	2.2	11.2	2.2
POL 1	3	533	4.6	0.9	5.0	0.9
POL 2	3	544	8.5	1.6	9.5	1.8
POL 3	3	546	5.6	1.0	9.0	1.6

b. *Linearity/assay reportable range:*

The linearity of the LD assay on the S40 Clinical Analyzer was evaluated using a method based on CLSI EP6-A. Commercial linearity standards with known ratios of enzymatic activity for LD (11 level) were used. The assigned value of the highest sample (11) was set to its mean value. The assigned values of the other levels were calculated by multiplying the mean value by the ratios obtained from the manufacturer of the standards.

The mean value of each set of quadruplicate measurements was calculated. The % recovery was calculated for each sample. The results are summarized below:

Sample	Assigned Value (U/L LD)	Measured Value (U/L LD)	% Recovery
1	10	10	99 %
2	53	58	109 %
3	96	106	110 %
4	144	157	109 %
5	192	207	108 %
6	288	294	102 %
7	384	395	103 %
8	432	436	101 %
9	480	490	102 %
10	576	572	99 %
11	672	672	100 % (assigned)

The results from linear regression analysis of the assigned value (x-axis) versus measured value (y-axis) are  $y = 0.990x + 9.3$ ,  $r^2 = 0.9992$ .

The S-Test LD assay demonstrates acceptable linearity from 10 to 672 U/L.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*  
 The S-Test LD cartridges are factory calibrated and traceable to the Japanese Certified Enzyme Reference Materials (JC ERM 20327). The 2-D barcode printed on each cartridge provides the analyzer with lot-specific calibration data.

Real time stability studies for the assay reagents have been conducted. Protocols and acceptance criteria were described and found to be acceptable. When stored at 2 - 8 °C the assay reagents are stable for 12 months.

- d. *Detection limit:*  
 The limit of blank (LoB) and limit of detection (LoD) studies were performed in accordance to CLSI EP17-A. These determinations were performed using 60 replicates of a true blank (BSA, 7.5% solution in saline) for the LoB and 60 replicates of five low level sample values for the LoD. The true blank results were ranked from lowest to highest. The LoB was calculated as the mean of the 57<sup>th</sup> and 58<sup>th</sup> highest values for the true blank. The standard

deviation of the 60 results for the low samples was calculated. The LoD was calculated using the following equation:

$$\text{LoD} = \text{LoB} + (1.645 * \text{SD low samples})$$

The estimate for LoB is 6.5 U/L

The estimate for LoD is 9 U/L.

*e. Analytical specificity:*

The interference studies were performed in accordance to CLSI EP7-A2. Various concentrations of the potential interfering compounds were added to aliquots of normal (approximately 100 U/L LD) and abnormal (approximately 400 U/L LD) serum pools. The control samples consisted of aliquots of the same serum pools diluted with an equivalent volume of diluent containing no interfering compound. All samples were tested in triplicate on the S40 Clinical Analyzer. The following interference studies were performed:

Study	Compound Used	Concentrations tested (mg/dL)
Bilirubin	Unconjugated bilirubin	up to 50
Hemolysis	Hemoglobin	up to 1000
Lipemia	Intralipid	up to 2000

Interference was considered significant if the analyte recovery changed by more than  $\pm 10\%$ . Analysis of the data demonstrated:

Bilirubin: No significant interference.

Hemolysis: Positive interference ( $\geq 20\%$ ) occurred at all levels tested.

Lipemia (Intralipid): No significant interference below 750 mg/dL.

Interference occurred (absorbance exceeds reaction limit) at 1000 mg/dL.

Other limitations:

- The product insert states that hemolyzed samples should not be used.
- The product insert refers to literature for a comprehensive list of drugs and other substances that can affect LD concentrations in serum.

Carryover: The sponsor evaluated the carryover characteristics of the proposed assay at three POLs and in-house using three serum samples (with normal, intermediate and elevated levels of LD). The % carryover and t-statistic were calculated for each lab. The carryover specifications for all four labs were met.

f. *Assay cut-off:*  
Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

In-House Studies:

The method comparison studies were performed in accordance to CLSI EP9-A2. Eighty one serum samples with LD values ranging from 26 to 652 U/L were evaluated in singlicate on the S40 Clinical Analyzer. For comparison, the same samples were evaluated in singlicate using the predicated device. Results from samples under or over the reportable range for either the proposed device or the predicate device were not included in the regression analyses. Least-squares regression analysis (Deming) yielded the following results:

n	Range (U/L LD)	Regression Equation	Correlation Coefficient	Std Error	Confidence Interval Slope	Confidence Interval Intercept
81	26 to 652	$Y = 0.971x - 5.3$	0.9857	19.8	0.934 to 1.008	-13.3 to 2.7

Consumer Studies:

Serum samples were evaluated in singlicate on the S40 Clinical Analyzer at four labs associated with physician offices. For comparison, the same samples were evaluated in singlicate using the predicated device. Results from samples under or over the reportable range for either the proposed device or the predicate device were not included in the regression analyses. Least-squares regression analysis (Deming) yielded the following results:

Lab	n	Range (U/L LD)	Regression Equation	Correlation Coefficient	Std Error	Confidence Interval Slope	Confidence Interval Intercept
A	51	27 - 633	$Y = 0.963x - 7.0$	0.9971	8.4	0.942 to 0.984	-11.1 to -2.8
B	55	26 - 652	$Y = 0.968x + 2.4$	0.9989	6.4	0.956 to 0.981	-0.4 to 5.1
C	55	26 - 652	$Y = 0.980x - 4.4$	0.9972	10.5	0.960 to 1.001	-9.0 to 0.2
D	55	26 - 652	$Y = 0.961x + 6.6$	0.9972	10.5	0.941 to 0.981	2.2 to 11.0

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:  
100 – 190 U/L from McPherson & Pinkus (Ed.), *Henry's Clinical Diagnosis and Management by Laboratory Methods*, 21<sup>st</sup> Edition, W.B. Saunders Co., Appendix 5 (2006).

Range was confirmed by testing 56 normal patients, ages 18-92 (27 males and 29 females) on the S-Test LD assay, with the following results:

Mean = 128 U/L (1 SD=22) range 88 – 181 U/L

The sponsor included the following statement in the product insert:

- This assay has not been evaluated in children (< 18 years old).

**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.