

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

A. 510(k) Number:

k072142

B. Purpose for Submission:

New device

C. Measurand:

Alkaline Phosphatase (ALP), Amylase (AMY), Aspartate Aminotransferase (AST)

D. Type of Test:

Quantitative

E. Applicant:

Alfa Wassermann Diagnostic Technology, Inc.

F. Proprietary and Established Names:

S Test Alkaline Phosphatase (ALP) Reagent cartridge
S Test Amylase (AMY) Reagent cartridge
S Test Aspartate Aminotransferase (AST) Reagent cartridge

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CKF-Alkaline Phosphatase	Class II	21 CFR § 862.1050	75-Chemistry
CIJ – Amylase	Class II	21 CFR § 862.1070	75-Chemistry
CIT- Aspartate Aminotransferase	Class II	21 CFR § 862.1100	75-Chemistry

H. Intended Use:

1. Intended use(s):

See Indications for Use below.

2. Indication(s) for use:

The S-Test Alkaline Phosphatase Reagent is intended for the quantitative determination of alkaline phosphatase activity in serum or heparin plasma using the S40 Clinical Analyzer. Measurements of alkaline are used in the diagnosis and treatment of liver, bone, parathyroid, and intestinal diseases. This test is intended for use in clinical laboratories or physicians office laboratories. For *in vitro* diagnostic use only.

The S-Test Amylase Reagent is intended for the quantitative determination of amylase activity in serum or heparin plasma using the S40 Clinical Analyzer. 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 S-Test Aspartate Aminotransferase Reagent is intended for the quantitative determination of aspartate aminotransferase activity in serum or heparin plasma using the S40 Clinical Analyzer. Aspartate aminotransferase measurements are used in the diagnosis and treatment of certain types of liver and heart disease. 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 prescription use only

4. Special instrument requirements:

S40 Clinical Analyzer

I. Device Description:

The ALP, AMY and AST are single use reagent cartridges having two reagent cells, Photometric reaction cuvette, film seal and a 2-D code label. The reagent cells contain the following reagents:

ALP Reagent 1 – ethyl amino ethanol buffer and magnesium chloride, Reagent 2 – p-nitrophenyl phosphate

AMY Reagent 1 – Sodium chloride, Calcium chloride and Good’s buffer, Reagent 2 – α -2-chloro-3-nitrophenyl galactopyranosyl maltoside, Potassium thiocyanate and Good’s buffer

AST Reagent 1 – Nicotinamide-adenine dinucleotide (reduced form), Malate dehydrogenase, 2-amino-2-hydroxymethyl-1,3-propanediol buffer and L-aspartic acid, Reagent 2 – L-aspartic acid α -ketoglutaric acid and 2-amino-2-hydroxymethyl-1,3-propanediol buffer.

J. Substantial Equivalence Information:

1. Predicate device name(s):

ACE plus ISE/Clinical Chemistry System, Alfa Wassermann
Piccolo xpress Chemistry Analyzer, Abaxis Inc.

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

k931786 and k950164 respectively

3. Comparison with predicate:

Alkaline Phosphatase (ALP):

The device and the predicate devices share a similar intended use, analytes measured, test principle, reaction type and sample type.

Differences			
Item	S40 Clinical Analyzer S Test ALP Reagent	ACE plus ISE Clinical Chemistry System	Piccolo xpress Chemistry Analyzer
Sample Volume	5 μ L	4 μ L	100 μ L
Measuring Range	20-896 U/L	2-1400 U/L	5-2400 U/L
Detection Limit	20 U/L	2 U/L	5 U/L

Amylase (AMY):

The device and the predicate devices share a similar intended use, analytes measured, test principle, reaction type and sample type.

Differences			
Item	S40 Clinical Analyzer S Test ALP Reagent	ACE plus ISE Clinical Chemistry System	Piccolo xpress Chemistry Analyzer
Sample Volume	8 µL	8 µL	100 µL
Measuring Range	8-1594 U/L	0-1900 U/L	5-4000 U/L
Detection Limit	8 U/L	0 U/L	5 U/L

Aspartate aminotransferase (AST):

The device and the predicate devices share a similar intended use, analytes measured, test principle, reaction type and sample type.

Differences			
Item	S40 Clinical Analyzer S Test ALP Reagent	ACE plus ISE Clinical Chemistry System	Piccolo xpress Chemistry Analyzer
Sample Volume	15 µL	14µL	100 µL
Measuring Range	8-354 U/L	7-450 U/L	3-1000 U/L
Detection Limit	8 U/L	7 U/L	5 U/L

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

- CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (2004)
- CLSI EP10-A: Preliminary Evaluation of Quantitative Clinical Laboratory Methods; Approved Guideline –Second Edition (2002)
- CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach: Approved Guideline (2003)
- CLSI EP7-A: Interference Testing in Clinical Chemistry; Approved Guideline (2002)
- CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (2004)
- CLSI EP9-A2: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (2002)
- CLSI C28-A2: How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline-Second Edition (2000), Section 8.2: Transference and Validation

L. Test Principle:

S Test ALP – Alkaline Phosphatase in the sample catalyzes the cleavage of p-nitrophenylphosphate substrate to form the visible product, p-nitrophenyl which is measured spectrophotometrically at 405 nm. The rate of increase in absorbance is directly proportional to the alkaline phosphatase activity in the sample.

S Test AMY – Amylase in the sample catalyses the cleavage of α -2-chloro-4-nitrophenol- α -galactopyranosyl maltoside substrate to form the visible product 2-chloro-p-nitrophenol which is measured spectrophotometrically at 405 nm. The rate of increase in absorbance is directly proportional to the α -amylase activity in the sample.

S Test AST – AST in the sample converts the L-aspartate and α -ketoglutarate in the reagent to oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD in malate dehydrogenase catalyzed indicator reaction. The rate of decrease in absorbance from NADH to NAD, monitored bichromatically at 340 nm/546 nm, is proportional to the AST activity in a sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were conducted in-house and at three Physician Office Laboratories (POL) (with three trained operators typically found in these settings) by testing three serum samples. The samples were run once a day, three times per run for five days using one instrument at each site. The results are presented below:

ALP U/L				
Lab	Sample	Mean	% CV	
			Within Run	Total
In-House	1	43	4.2%	4.7%
POL 1	1	44	2.3	5.0%
POL 2	1	45	4.0%	4
POL 3	1	41	2.9%	2.9%
In-House	2	136	1.8%	2.4%
POL 1	2	142	2.4%	4.0%
POL 2	2	144	2.6%	2.6%
POL 3	2	137	1.5%	1.5%
In-house	3	733	4.4%	4.4%
POL 1	3	783	3.6%	3.6%
POL 2	3	768	3.7%	4.1%
POL 3	3	796	2.3%	2.3%

AMY U/L				
Lab	Sample	Mean	%CV	
			Within Run	Total
In-House	1	47	4.7%	4.7%
POL 1	1	48	2.9%	3.3%
POL 2	1	48	6.5%	6.7%
POL 3	1	49	3.5%	3.9%
In-House	2	170	1.1%	1.1%
POL 1	2	172	4.3%	4.6%
POL 2	2	178	1.2%	1.2%
POL 3	2	175	3.2%	3.2%
In-House	3	1424	2.7%	2.7%
POL 1	3	1491	3.2%	3.2%
POL 2	3	1520	1.0%	1.0%
POL 3	3	1527	1.4%	1.7%

Lab	AST U/L			
	Sample	Mean	%CV	
			Within Run	Total
In-House	1	26	1.9%	2.0%
POL 1	1	26	4.6%	7.0%
POL 2	1	27	4.8%	4.7%
POL 3	1	27	3.7%	3.8%
In-House	2	142	2.0%	2.8%
POL 1	2	145	1.1%	1.1%
POL 2	2	145	1.2%	1.2%
POL 3	2	146	1.0%	1.3%
In-House	3	306	2.2%	2.5%
POL 1	3	307	1.1%	1.3%
POL 2	3	312	1.0%	1.3%
POL 3	3	313	0.8%	0.9%

b. *Linearity/assay reportable range:*

Linearity across the assay range was confirmed by testing commercial linearity standards, 5-11 levels each with known commercial concentrations of ALP, AMY and AST. Each level was tested in replicates of four. Results are presented below:

ALP U/L	Assigned Value	Measured Value	% Recovery
Sample 1	28	27	96%
Sample 2	53	55	104%
Sample 3	78	82	105%
Sample 4	104	108	104%
Sample 5	128	131	98%
Sample 6	133	131	98%
Sample 7	255	262	103%
Sample 8	264	265	100.0%
Sample 9	511	517	101%
Sample 10	724	744	103%
Sample 11	896	896	100%
Linear Regression – $y = 1.009x + 1.17$ $r^2 = 0.999$			

AMY U/L	Assigned Value	Measured Value	% Recovery
Sample 1	17	18	104%
Sample 2	177	181	102%
Sample 3	354	363	102%
Sample 4	708	735	104%
Sample 5	885	932	105%
Sample 6	1240	1277	103%
Sample 7	1594	1594	100%
Linear Regression – $y = 1.011 x + 10.1, r^2 = 0.999$			

AST U/L	Assigned Value	Measured Value	% Recovery
Sample 1	7.0	8	112.1%
Sample 2	71	70	100.6%
Sample 3	141	140	100.6%
Sample 4	283	281	99.9%
Sample 5	354	354	100%
Linear Regression – $y = 0.997x - 0.29, r^2 = 0.999$			

The reportable ranges are 20-896 U/L for ALP, 8-1594 U/L for AMY and 8-354 U/L for AST.

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

The S Test ALP, S Test AMY and S Test AST cartridges are factory calibrated and traceable to the Japanese Certified Enzyme Reference Materials (JC ERM 20327 ALP, AMY) and (JCCLS CRM 003a AST). The 2-D barcode printed on each cartridge provides the analyzer with lot-specific calibration data.

Real time stability studies have been conducted. Protocols and acceptance criteria were described and found to be acceptable. When stored at 2-8 °C the assay reagent is good until the expiration date on the label.

d. Detection limit:

The Limit of Blank and Limit of Detection were determined for each analyte by running a low sample and a BSA sample, (7.5% in saline) for 3 days, 20 replicates/day for a total of 60 results. The testing was split between two instruments. The detection limits were determined to be 20 U/l for ALP, 8 U/L for AMY and 8 U/L for AST.

e. Analytical specificity:

Interference studies to determine the effects of Unconjugated Bilirubin, Hemolysis and Lipemia were performed. Seven serum pool containing approximately 62 U/L ALP, 66 U/L AMY and 17 U/L AST were spiked with various concentrations of unconjugated bilirubin (0-50 mg/dL), hemoglobin (0-1000 mg/dL) and Intralipids (0-2000 mg/dl). Sponsor states that interference is considered to be significant if the analyte recovery changes by more than 10%.

ALP – There was no significant interference (no change greater than 10%) from bilirubin or lipemia at the tested concentrations. Hemolysis showed a positive interference of (~15%) at 31 mg/dL. Any level of hemolysis may cause interference. The sponsor states that users should not use hemolyzed specimens.

AMY - There was no significant interference (no change greater than 10%) from bilirubin or Lipemia at the tested concentrations. Hemolysis showed a positive interference of (~17%) 31 mg/dL. Any level of hemolysis may cause interference. The sponsor states that users should not use hemolyzed specimens.

AST – There was no significant interference (no change greater than 10%) for Lipemia at the tested concentrations. Positive interference (12-17%) occurred at 1.6, 3.1, 6.3 and 12.5 mg/dL bilirubin, the four lowest levels tested. Any level of icterus may cause interference. The sponsor states that users should not use icteric specimens. Hemolysis showed a positive interference (>14%) occurred at all levels tested. The sponsor states that users should not use hemolyzed specimens.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Clinical correlation studies were performed comparing the S-Test ALP, AMY and AST results generated on the S40 Clinical analyzer against the results from the ACE Clinical analyzer using totaling of 180 ALP samples, 196 AMY samples and 177 AST samples. Of the 180 ALP serum samples (15 were diluted and 17 were spiked samples), 196 AMY serum samples (20 were diluted and 29 were spiked samples) and 177 AST serum samples (13 were diluted and 27 were spiked samples). All the samples were measured in singlet. The correlation study between the device and the predicate yielded the following results

Test	n	Slope	Intercept	r	Sample range (U/L)
S Test ALP	182	0.969	-1.2	0.997	28 - 733
S Test AMY	196	0.969	3.19	0.997	9 - 1461
S Test AST	177	1.014	-0.83	0.998	10 - 333

Performance for the S Test ALP, S Test AMY and S Test AST was evaluated at four Physician Office Laboratories and with a total of four operators. Operators ran for ALP 46 unaltered clinical serum samples and 8 diluted samples, AMY 41 unaltered clinical samples, 10 diluted and 12 spiked samples and AST 41 unaltered clinical samples, 8 diluted and 12 spiked samples. The S Test ALP, AMY and AST test results were compared to the ACE results. All operators had experience running chemistry instruments. The correlation study between the device and the predicate for serum yielded the following results.

		n	Slope	Intercept	r	Range (U/L)
ALP	Lab A	54	0.990	2.2	0.998	21-848
	Lab B	55	0.987	10.6	0.996	21-848
	Lab C	52	0.991	1.1	0.998	21-848
	Lab D	54	0.987	4.3	0.998	21-848
AMY	Lab A	61	0.94	5.1	0.997	20-1565
	Lab B	61	0.94	7.6	0.997	20-1565
	Lab C	60	0.94	3.7	0.997	20-1565
	Lab D	63	0.93	10.2	0.997	20-1565
AST	Lab A	60	1.06	2.9	0.998	10-317
	Lab B	61	1.08	-2.1	0.998	10-317
	Lab C	60	1.07	-0.8	0.998	10-317
	Lab D	60	1.09	-5.5	0.998	10-317

b. *Matrix comparison:*

A serum / plasma comparison test was performed for the S-Test ALP, S-Test AMY and S-Test AST assays. Paired samples were assayed on the S40 System. Samples were spiked with the analyte to help cover the assay range. The correlation is as follows:

ALP - $y = 0.921x + 3.8$, $r = 0.997$, range 49-847 U/L, $n=30$

AMY - $y = 1.028x - 6.2$, $r = 0.996$, range 31-292 U/L, $n = 32$

AST - $y = 0.994x + 5.2$, $r = 0.998$, range 10-351 U/L, $n = 31$

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:

Eighty-one (81) normal serum samples for ALP, AMY and AST were evaluated on the S40 Clinical Analyzer to determine if the reference range of the predicates (ACE Clinical Analyzer and Piccolo) could be transferred to the new assays. The sponsors' acceptance criterion is 90% of the assay results for the normal samples are within the predicate range. Analysis confirmed sufficient agreement (7.4% ALP, 4.8% AMY and 8.6% AST non-congruent results, sponsor specification $\leq 10\%$) to transfer the reference range.

ALP – 35-123 U/L

AMY – 25-125 U/L

AST - 11-38 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.