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

A. 510(k) Number:

k130141

B. Purpose for Submission:

New Device

C. Measurand:

Alkaline phosphatase (ALP)

D. Type of Test:

Quantitative, enzymatic activity

E. Applicant:

Hitachi Chemical Diagnostics

F. Proprietary and Established Names:

S TEST Reagent Cartridge Alkaline Phosphatase (ALP)

G. Regulatory Information:

1. Regulation section:

   21 CFR § 862.1050 Alkaline Phosphatase (ALP) or isoenzymes test system

2. Classification:

   Class II

3. Product code:

   CJE

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:
   The S TEST Reagent Cartridge Alkaline Phosphatase (ALP) is intended for the quantitative measurement of alkaline phosphatase activity in serum, lithium heparinized plasma, or sodium citrate plasma using the HITACHI Clinical Analyzer. The S TEST Reagent Cartridge Alkaline Phosphatase (ALP) is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

   Measurements of alkaline phosphatase are used in the diagnosis and treatment of liver, bone, parathyroid, and intestinal diseases.

3. Special conditions for use statement(s):

   For prescription and point-of-care use.

4. Special instrument requirements:

   Hitachi Clinical Analyzer E40

I. Device Description:

The S TEST reagent cartridges for the Hitachi Clinical Analyzer E40 are made of plastic and include two small reservoirs capable of holding two separate reagents
R1 (2-Ethylaminoethanol Buffer and Magnesium Chloride) and R2 (p-Nitrophenyl phosphate disodium salt and 2-Amino-2-hydroxymethyl-1,3-propanediol Buffer), separated by a reaction cell/photometric cuvette. The cartridges also include a dot code label that contains all chemistry parameters, calibration factors, and other production-related information, e.g., expiration dating.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Roche Cobas c systems ALP2

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

   k100853

3. Comparison with predicate:

	Hitachi S Test Reagent Cartridge ALP (Candidate Device)	Roche Cobas c systems ALP2 (Predicate Device, k100853)
Similarities		
Intended Use/ Indications for Use	For the quantitative measurement of alkaline phosphatase activity in serum, lithium heparinized plasma, or sodium citrate plasma Measurements of alkaline phosphatase are used in the diagnosis and treatment of liver, bone, parathyroid, and intestinal diseases.	Same
Specimen Type	Human serum or (sodium citrate or lithium heparin) plasma	Same
Differences		
Test Principle	In the presence of magnesium ions, ALP reacts with p-NPP to release p-nitrophenol.	In the presence of magnesium and zinc ions, p-NPP is cleaved by phosphatases into phosphate and p-nitrophenol
Testing Environment	Physician office or clinical lab	Clinical lab
Detection Wavelength	405/508 nm	480/450 nm
Claimed measuring range	10 to 1,000 U/L	5 to 1,200 U/L
Detection Limit	1.8 U/L	5 U/L

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

CLSI/NCCLS EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; 2004

CLSI/NCCLS EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach; 2003

CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline, 2005

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; 2004

L. Test Principle:

Alkaline phosphatase (ALP) in the sample reacts with its substrate, p-nitrophenyl phosphate (p-NPP), in ethylaminoethanol (EAE) buffer, to release p-nitrophenol (yellow). The ALP activity is determined by measuring the rate of p-nitrophenol production. This rate of change in absorbance is directly proportional to the ALP activity in the serum.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

20-day In-house Precision: The studies followed CLSI EP5-A2, where three levels of archived (stored frozen) patient serum samples were each tested in two runs, twice a day, for 20 days on the Hitachi E40 Clinical Analyzer. The results were as follows:

ALP- Low, Level 1, Summary

ALP	Within-Run	Total
Mean (U/L)	42.7	42.7
SD (U/L)	1.3	2.25
%CV	3.1%	5.3%

ALP- Middle, Level 2, Summary

ALP	Within-Run	Total
Mean (U/L)	80.5	80.5
SD (U/L)	3.07	4.65
%CV	3.8%	5.8%

ALP- High, Level 3, Summary

ALP	Within-Run	Total
Mean (U/L)	555.6	555.6
SD (U/L)	13.99	24.69
%CV	2.5%	4.4%

Point-of-Care Precision:

Three levels of samples A (low), B (middle), and C (high) were tested by three POL sites, six times a day for five days on the Hitachi E40 Clinical Analyzer. The samples were native (neat) serum specimens (stored frozen). The precision estimates are described below:

Site #	Sample	Mean (U/L)	Within-run Precision		Total Precision	
			SD (U/L)	%CV	SD (U/L)	%CV
1	A	13.2	0.80	6.1	0.96	7.3
2	A	12.8	0.89	6.9	1.00	7.8
3	A	13.4	1.18	8.8	1.23	9.2
1	B	76.2	1.22	1.6	2.26	3.0
2	B	68.8	4.39	6.4	5.24	7.6
3	B	70.0	4.56	6.5	6.28	9.0
1	C	408.6	4.22	1.0	26.01	6.4
2	C	390.1	23.21	6.0	23.21	6.0
3	C	402.9	13.21	3.3	12.33	3.1

b. Linearity/assay reportable range:

A linearity study was conducted based on the CLSI EP6-A guideline by comparing observed versus expected values for 10 samples. Ten (10) serial dilutions (4 to 1168 U/L were prepared and tested. The dilutions were prepared using a commercial linearity/calibration set. The calibration samples were assigned their reference values arithmetically from the labeled values and were tested in duplicate by the Hitachi E40 Clinical Analyzer. The mean Hitachi results (y-axis) were plotted against the expected values (x-axis). The parameters of linear regression are as follows:

$$y = 0.9651x + 3.6328$$
$$R^2=0.999$$

The ALP assay is linear between 4 U/L and 1106 U/L. The results of the study support the sponsor's claim that the Hitachi S Test ALP Reagent Cartridge is linear across the measuring range of 10 to 1,000 U/L.

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

Each lot of S TEST ALP cartridges is calibrated by the manufacturer prior to shipment using material traceable to Japanese Enzyme Reference Material (JC-ERM). The barcode printed on each cartridge provides the analyzer with lot-specific calibration data. Alkaline Phosphatase concentration is directly determined by multiplying the change in absorbance of the unknown sample by the calibrator factor on the barcode. No user calibration is needed.

Commercially available controls are required but not provided. Users should follow federal, state, and local regulatory requirements regarding quality control practices.

d. Detection limit:

Detection limit studies were performed according to CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation. The blank sample for the reagent system was assayed on the Hitachi Clinical Analyzer E40, 20 times per day for three days for a total of 60 replicate results to determine LOB. Five low samples were assayed on the Hitachi Clinical Analyzer E40, 4 times per day for three days for a total of 60 replicate results with the specific reagent cartridges to determine LOD. Each of three samples targeted at 10 U/L were assayed 6 times per day for three days on three different analyzers for a total of 54 replicate results to determine LoQ. The LoQ was determined based on inter-assay precision of < 15% CV.

Analyte	LoB (U/L)	LoD (U/L)	LoQ (U/L)
Alkaline Phosphatase	1.02	1.8	10

The measuring range of the assay was 10 to 1000 U/L.

e. Analytical specificity:

Interference studies were performed according to CLSI EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline) were performed to determine the effects from potential interferents. Two levels of commercial control sera (ALP low and high, approximately 35 U/L and 100U/L) were spiked to six levels with each interferent, and all seven samples (the 6 spiked samples and the neat, zero baseline sample) were tested in replicates of three on the Hitachi E40 Clinical Analyzer. In each case, the spiked sample result mean was compared to its neat control mean result, and recoveries were calculated. The sponsor claims non significant interference (≤10% difference) for the substances and concentrations listed in the table below.

Alkaline Phosphatase

Interferent Compound	Highest Concentration Showing No Interference
Hemoglobin	500 mg/dL
Unconjugated bilirubin	50 mg/dL
Lipemia	2,000 mg/dL
Ascorbic acid	50 mg/dL

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:

In-house method comparison study was conducted using a total of 97 clinical specimens (4 diluted and 7 spiked) spanning the dynamic range (11 to 926 U/L), samples were assayed on the Hitachi Clinical Analyzer E40 in singleton on both the Hitachi S Test Reagent and the Roche Cobas c systems ALP2 (predicate device). The comparative data were analyzed by linear regression and are shown below.

Internal study summary – ALP (U/L)

n	Hitachi Range	Regression Equation	"r"	95% CI Slope	95% CI Intercept
97	11 to 926 U/L	y = 0.926x + 4.8075	0.996	(0.909 to 0.943)	(-0.2 to 9.8)

External site method comparison study: A series of approximately 70 serum specimens (4 diluted) with ALP values ranging from 11 to 745 U/L were assayed on the Hitachi Clinical Analyzer E40 at three sites using S TEST Reagent Cartridge ALP (y) and Roche Cobas c systems ALP2 (predicate device) as the reference method (x). The samples were native serum specimens. Linear regression analysis yielded the following results:

POL study summary- ALP (U/L)

Site #	n	Sample Range	Regression Equation	"r"	95% CI Slope	95% CI Intercept
1	77	13 to 745 U/L	y=0.967x +2.9	0.99	0.949 to 0.984	-0.6 to 6.4
2	72	11 to 688 U/L	y=0.942x -0.7	0.99	0.925 to 0.960	-4.4 to 2.9
3	72	12 to 736 U/L	y=0.980x +0.8	0.99	0.961 to 0.999	-3.2 to 4.7

b. Matrix comparison

A study was performed to validate the use of two plasma types as an alternative to serum for the Hitachi Clinical Analyzer E40 with S TEST Reagent Cartridge ALP. The plasma types were sodium citrate and lithium heparin. Thirty-eight (38) matched serum/plasma samples that spanned the range of the assay (13 to 967 U/L) were assayed in singleton. The study set included five diluted samples and six spiked samples. The results were compared using linear regression (plasma = y-axis, each type). The performance characteristics were as follows.

N = 38
Range (serum) = 13 to 967 U/L

	Sodium Citrate Plasma	Heparinized Plasma
Slope (95% CIs)	1.03 (1.01 to 1.05)	1.01 (1.00 to 1.02)
y-intercept (95% CIs)	-11.2 (-15.5 to -7.0)	-5.4 (-8.3 to -2.6)
r	0.999	0.999

The sponsor claimed that lithium heparin and sodium citrate are acceptable anti-coagulants to be used with the ALP assay.

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:

	Males	Females
ALP Reference Range[1]	53 to 128 U/L	42 to 141 U/L

[1] Tietz Fundamentals of Clinical Chemistry, 4th Edition, WB Saunders Company, (1996)

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.