510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K223317

B Applicant

Abbott Ireland Diagnostics Division

C Proprietary and Established Names

Alkaline Phosphatase2

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
CJE	Class II	21 CFR 862.1050 - Alkaline Phosphatase Or Isoenzymes Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Alkaline Phosphatase

C Type of Test:

Quantitative, photometric/colorimetric

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Alkaline Phosphatase2 assay is used for the quantitation of alkaline phosphatase in human serum or plasma on the ARCHITECT c System. Measurements of alkaline phosphatase or its isoenzymes are to be used as an aid in the diagnosis and treatment of liver, bone, parathyroid, and intestinal diseases.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

ARCHITECT c8000

IV Device/System Characteristics:

A Device Description:

The Alkaline Phosphatase2 assay kit consists of two ready to use reagent solutions, R1 and R2.

R1 is a buffer solution containing 179.550 g/L of 2-amino-2-methylpropanol(AMP) and sodium azide as a preservative. R2 is a substrate solution containing 30.430 g/L of 4-nitrophenyl phosphate and sodium azide as a preservative.

The Alkaline Phosphatase2 assay has two approaches to calculate results, i.e., calibration method and calibration factor method. The user decides which of the two methods to use. The calibration factor method allows users to perform testing without using a physical calibrator. There are separate assay parameter files that user must upload to the instrument for use with each method.

- The calibration method utilizes the linear data reduction method based on the Consolidated Chemistry Calibrator to generate a calibration and results.

- The calibration factor method utilizes the factor data reduction method to generate a calibration and results. The calibration factor for the Alkaline Phosphatase2 assay is 1931.

B Principle of Operation:

Alkaline Phosphatase in a sample catalyzes the hydrolysis of colorless para-nitrophenyl phosphate (*p*-NPP) to give para-nitrophenol (yellow phenoxide form at alkaline pH) and inorganic phosphate.

The rate of absorbance increase at 404 nm is directly proportional to the alkaline phosphatase activity in the sample. Optimized concentrations of zinc and magnesium ions are present to activate the alkaline phosphatase in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Alkaline Phosphatase

B Predicate 510(k) Number(s):

K023807

C Comparison with Predicate(s):

Device & Predicate Device(s):	K223317	K023807
Device Trade Name	Alkaline Phosphatase2	Alkaline Phosphatase
General Device Characteristic Similarities		
Intended Use/Indications For Use	The assay is used for the quantitation of alkaline phosphatase in human serum or plasma.	Same
Specimen Type	Human serum or plasma	Same
General Device Characteristic Differences		
Standardization	IFCC traceable	Molar extinction of p-nitrophenol (non-IFCC method)
Calibration Method	Calibration and Calibration Factor method	Factor method
Assay Range	Analytical Measuring Interval: 4-4522 U/L	Analytical Measuring Interval: 5-4555 U/L
Lower Limits of Measurement	Limit of Blank: 1 U/L Limit of Detection: 3 U/L Limit of Quantification: 4 U/L	Limit of Detection: 5 U/L Limit of Quantification: 5U/L

VI Standards/Guidance Documents Referenced:

Clinical and Laboratory Standards Institute (CLSI) EP05-A3: Evaluation of Precision of Quantitative Measuring Procedures-Third Edition.

CLSI EP06: Evaluation of the Linearity of Quantitative Measurement Procedure.- Second Edition.

CLSI EP07: Interference Testing in Clinical Chemistry- Third Edition.

CLSI EP09-A3: Measurement Procedure Comparison and Bias Estimation using Patient Samples-Third Edition.

CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures-Second Edition.

CLSI EP 37: Supplemental Tables for Interference Testing in Clinical Chemistry.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

The sponsor has validated the analytical performance of the candidate device using both the calibration method and the calibration factor method.

1. Precision/Reproducibility:

The precision study was performed according to the CLSI EP05-A3 guideline. Three (3) instruments and three (3) lots of reagents. Two (2) controls and four (4) human serum panels were tested in a minimum of two (2) replicates, twice (2) per day for twenty (20) days for a minimum of 80 replicates per sample per each unique instrument/reagent/calibrator combination. The results of the precision studies are summarized below. The within-laboratory SD and %CV includes within-run, between-run, and between-day variance components.

Calibration Method

Specimen	Reagent/ Instrument combination	N	Mean (U/L)	Repeatability		Within-Laboratory	
				SD	%CV	SD	%CV
Control Level 1	1	80	115	0.8	0.7	2.7	2.3
	2	80	116	0.8	0.7	3.0	2.6
	3	80	115	1.0	0.8	2.9	2.6
Control Level 2	1	80	430	1.2	0.3	7.5	1.7
	2	80	428	1.4	0.3	7.8	1.8
	3	80	428	1.7	0.4	8.4	2.0
Sample A	1	80	10	0.8	8.1	0.8	8.7
	2	80	9	0.6	6.3	0.6	6.7
	3	80	9	1.1	11.7	1.1	11.8
Sample B	1	80	42	0.8	2.0	1.2	2.9
	2	80	42	0.7	1.6	1.0	2.3
	3	80	41	0.9	2.3	1.2	2.9
Sample C	1	80	2135	6.3	0.3	40.5	1.9

Specimen	Reagent/ Instrument combination	N	Mean (U/L)	Repeatability		Within-Laboratory	
				SD	%CV	SD	%CV
	2	80	2045	6.8	0.3	43.6	2.1
	3	80	2143	13.3	0.6	42.3	2.0
Sample D	1	80	4430	18.7	0.4	93.5	2.1
	2	80	4306	14.6	0.3	77.7	1.8
	3	80	4449	28	0.6	98.7	2.2

Calibration Factor Method

Specimen	Reagent/ Instrument combination	N	Mean (U/L)	Repeatability		Within-Laboratory	
				SD	%CV	SD	%CV
Control Level 1	1	80	116	0.8	0.7	2.7	2.3
	2	80	114	0.9	0.8	3.0	2.6
	3	80	116	0.9	0.8	3.1	2.6
Control level 2	1	80	435	1.2	0.3	7.5	1.7
	2	80	423	1.3	0.3	7.9	1.9
	3	80	434	1.7	0.4	8.3	1.9
Sample A	1	80	10	0.8	8.5	0.8	8.7
	2	80	9	0.7	7.2	0.7	7.2
	3	80	9	1.0	11.3	1.1	11.5
Sample B	1	80	42	0.7	1.6	1.1	2.7
	2	80	41	0.7	1.7	0.9	2.1
	3	80	41	0.9	2.2	1.2	2.9
Sample C	1	80	2158	6.4	0.3	39.5	1.8
	2	80	2017	6.7	0.3	44.9	2.2
	3	80	2172	13.4	0.6	40.1	1.8
Sample D	1	80	4478	18.8	0.4	91.9	2.1
	2	80	4248	14.4	0.3	81.0	1.9
	3	80	4508	28.5	0.6	94.5	2.1

The reproducibility study was performed according to the CLSI EP05-A3 guideline. Testing was conducted using one (1) lot of the Alkaline Phosphatase2 reagent, one (1) lot of the Consolidated Chemistry Calibrator, one (1) lot each of two (2) commercially available control sets, and three (3) instruments. Each instrument was operated by a different technician and each technician prepared an individual sample set. Five levels of controls were tested in three (3) replicates at two (2) separate times per day on five (5) different days. Reproducibility study results are summarized below:

Calibration Method

Specimen	N	Mean[a] (U/L)	Repeatability		Within-Laboratory[b]		Reproducibility[c]	
			SD	%CV	SD	%CV	SD	%CV
Control Level 1	90	113	1.1	1.0	2.6	2.3	2.6	2.3
Control level 2	90	460	2.6	0.6	5.6	1.2	6.6	1.4
Control Level A	90	71	0.8	1.2	0.9	1.3	1.1	1.5
Control Level B	90	177	1.7	0.9	4.4	2.5	4.4	2.5
Control Level C	90	359	2.1	0.6	6.3	1.8	7.0	2.0

[a]The values provided are the mean values across all instruments.
[b]Includes within-run, between-run, and between-day variability
[c]Includes within-run, between-run, between-day, and between-instrument variability

Calibration Factor Method

Specimen	N	Mean[a] (U/L)	Repeatability		Within-Laboratory[b]		Reproducibility[c]	
			SD	%CV	SD	%CV	SD	%CV
Control Level 1	90	108	1.0	0.9	2.4	2.3	2.7	2.5
Control level 2	90	443	2.6	0.6	5.4	1.2	8.0	1.8
Control Level A	90	68	0.8	1.2	0.9	1.3	1.5	2.2
Control Level B	90	171	1.6	0.9	4.4	2.6	5.2	3.0
Control Level C	90	345	2.1	0.6	6.1	1.8	8.9	2.6

2. Linearity:

Linearity studies were performed according to the CLSI EP06-2nd edition guideline. Fourteen (14) levels of samples were prepared by mixing different portions of a high sample and a blank sample. Each sample was tested in four (4) replicates on a single instrument using a single reagent lot in a single run. The results were analyzed using weighted least squares linear regression analysis. The results of the linearity regression analysis of the data are summarized below:

Calibration Method

Test	Slope	Intercept	R	Range Tested	Claimed Analytical Measuring Range
Alkaline Phosphatase	1.0181	NA*	0.9999	0-4978 U/L	4-4522 U/L

Calibration Factor Method

Test	Slope	Intercept	R	Range Tested	Claimed Analytical Measuring Range
Alkaline Phosphatase	1.0221	NA*	1.000	0-4978 U/L	4-4522 U/L

*NA: Model is forced through zero

3. Analytical Specificity/Interference:

The analytical specificity of the Alkaline Phosphatase2 assay on the ARCHITECT c8000 was established by conducting interference testing following the recommendations in the CLSI EP07 third edition guideline.

Interference from endogenous and exogenous substances was assessed using two serum samples with alkaline phosphatase concentration of 70U/L and 200 U/L. Each sample was further divided into two aliquots: a test sample (with added interferent) and a control sample (with no added interferent). Each sample was tested in ten (10) replicates using one (1) instrument and one (1) reagent lot. The difference and % difference between the mean concentration of the test and control sample were calculated.

The following tables list the highest concentration of each substance at which no significant interference was found, defined as a difference of less than or equal to ±10% between the test sample and control.

Potentially Interfering Endogenous Substances

Substances	Highest level tested that demonstrated no significant interference
Bilirubin - conjugated	15 mg/dL
Bilirubin - unconjugated	20 mg/dL
Hemoglobin	250 mg/dL
Total protein	15 g/dL
Triglycerides	1500 mg/dL

Potentially Interfering Exogenous Substances

Substances	Highest level tested that demonstrated no significant interference
Acetaminophen	160 mg/L
Acetylcysteine	150 mg/L
Acetylsalicylic acid	30 mg/L
Ampicillin-Na	80 mg/L
Ascorbic acid	60 mg/L

Substances	Highest level tested that demonstrated no significant interference
Biotin	4250 ng/mL
Ca-dobesilate	60 mg/L
Cefotaxime	60 mg/L
Cefoxitin	6600 mg/L
Cyclosporine	2 mg/L
Desacetylcefotaxime	6 mg/L
Doxycycline	20 mg/L
Ibuprofen	220 mg/L
Levodopa	8 mg/L
Magnesium sulfate	50 mg/L
Methyldopa	25 mg/L
Metronidazole	130 mg/L
Phenylbutazone	330 mg/L
Rifampicin	50 mg/L
Sodium heparin	4 U/mL
Theophylline (1,3-dimethylxanthine)	60 mg/L

Bilirubin conjugated (40 mg/dL), bilirubin unconjugated (40 mg/dL and 60 mg/dL) and hemoglobin (1000 mg/dL) were found to interfere at alkaline phosphatase levels of 70 U/L and 200 U/L. The following interference information was added to the labeling.

Potentially Interference Substance	Interference Level		Analyte Level		%Interference (95%CI)
	Default Unit	Alternative Units	Default Unit	Alternative Units	
Bilirubin-Conjugated	40 mg/dL	474 µmol/L	70 U/L	1.17 µkat/L	28% (27%, 29%)
Bilirubin Conjugated	40 mg/dL	474 µmol/L	200 U/L	3.33 µkat/L	11% (10%, 11%)
Bilirubin Unconjugated	40 mg/dL	684 µmol/L	70 U/L	1.17 µkat/L	21% (20%, 22%)
Bilirubin – Unconjugated	60 mg/dL	1026 µmol/L	200 U/L	3.33 µkat/L	10% (10%, 11%)
Hemoglobin	1000 mg/dL	10g/L	70 U/L	1.17 µkat/L	-33% (-34%, -31%)
Hemoglobin	1000 mg/dL	10 g/L	200 U/L	3.33 µkat/L	-13% (-14%, -13%)

The following limitation information is included in the Alkaline Phosphatase 2 reagent package insert:

"Specimens from patients undergoing alkaline phosphatase replacement therapy (asfotase alfa) may exhibit positive interference with alkaline phosphatase assay."

"Neonatal specimens have not been validated with this assay and should not be used."

"Specimens with conjugated bilirubin levels greater than 15 mg/dL or unconjugated bilirubin greater than 20 mg/dL may cause falsely elevated results with the Alkaline Phosphatase2 assay. Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity, Interference section of this package insert. To estimate sample icterus levels, refer to the Sample Interference Indices instructions for use to assess hemolysis (H), icterus (I) and lipemia (L) (HIL) indices."

"Specimens with Hemoglobin Levels greater than 250 mg/dL may cause falsely depressed results with the Alkaline Phosphatase2 assay. Refer to the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity, Interference section of this package insert. To estimate sample hemoglobin levels, refer to the Sample Interference Indices instructions for use to assess hemolysis (H), icterus (I) and lipemia (L) (HIL) indices."

4. Assay Reportable Range:

   4 – 4522 U/L

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   The Alkaline Phosphatase2 assay is traceable to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference method.

6. Detection Limit:

   Detection capability studies were conducted following the recommendations in CLSI EP17-A2. Studies were conducted using three (3) lots of the Alkaline Phosphatase2 reagents on each of 2 instruments (i.e., 6 conditions) over a minimum of three (3) days using both the calibration method and calibration factor method.

   The limit of blank (LoB) was evaluated and determined by running a zero-analyte sample. The limit of detection (LoD) and the limit of quantitation (LoQ) were determined using low-analyte samples. Each sample was measured in at least 60 replicates using each of three lots of reagents and two instruments (total of six instrument and reagent lot combinations). The highest LoB, LoD, and LoQ estimate from the six instrument and reagent lot combinations was used as the LoB, LoD and LoQ for the assay.

   The LoB was determined non-parametrically as the 95^{th} percentile from n=60 replicates of the zero-target samples. LoD was analyzed using a parametric data analysis method where the lowest concentration at which the analyte can be detected with 95% probability based on n=60 replicates of low-analyte level samples is considered the LoD. The LoQ was defined as the lowest concentration of analyte which has imprecision less than or equal to 20% CV. The results are summarized in the table below.

Calibration Method

	LoB	LoD	LoQ	AMI
Serum	1 U/L	3 U/L	4 U/L	4 to 4522 U/L

Calibration Factor Method

	LoB	LoD	LoQ	AMI
Serum	1 U/L	3 U/L	4 U/L	4 to 4522 U/L

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed following CLSI EP09-A3. The accuracy of the Alkaline Phosphatase2 assay on the ARCHITECT c8000 for serum samples was evaluated for agreement with the predicate device.

In this study, a total of 145 serum samples were tested and less than 10% of the samples were prepared by spiking or diluting specimens. The data were analyzed by Passing-Bablok regression using the first replicate of the candidate device results compared to the mean of the duplicate results from the comparator device. Method comparison study results are summarized below.

Calibration Method

N	Correlation Coefficient [95% CI]	Intercept [95% CI]	Slope [95% CI]	Concentration Range (per predicate) (U/L)
145	1.00 [0.99, 1.00]	-1 [-4, 0]	1.07 [1.06, 1.07]	8 – 4534

Calibration Factor Method

N	Correlation Coefficient [95% CI]	Intercept [95% CI]	Slope [95% CI]	Concentration Range (per predicate) (U/L)
143	1.00 [1.00, 1.00]	-2 [-3, 0]	1.08 [1.07, 1.08]	8 – 4042

2. Matrix Comparison:

A matrix equivalency study was conducted to support use of the Alkaline Phosphatase2 assay with the following additional specimen matrix types claimed in the product labeling: serum (separator tubes), lithium heparin plasma, lithium heparin (separator tubes) plasma and sodium heparin plasma. In the study, matched venous specimens were collected into each anticoagulant tube (i.e., evaluation tubes) and the serum control tube.

Each specimen was tested using one lot of reagents and one ARCHITECT c8000 instrument. The first replicate of the evaluation tubes was compared to the mean of the control serum measurements. A Passing-Bablok evaluation was performed, and results are summarized below:

Calibration Method:

Collection Tube	N	Min*	Max*	r	Slope (95% CI)	Intercept (95% CI)
Lithium heparin	63	34	3627	1.00	1.00 (0.99,1.01)	-2.59 (-3.24, -1.85)
Lithium heparin (separator tube)	63	33	3631	1.00	1.00 (0.99,1.01)	-1.93 (-2.68, -1.12)
Serum (separator tube)	63	36	3577	1.00	1.00 (0.99, 1.01)	0.33 (-0.42,1.20)
Sodium heparin	63	34	3581	1.00	0.99 (0.99, 1.00)	0.47 (-0.17, 1.38)

Calibration Factor Method:

Collection Tube	N	Min*	Max*	r	Slope (95% CI)	Intercept (95% CI)
Lithium heparin	63	34	3641	1.00	1.00 (1.00,1.01)	-2.00 (-2.95, -1.77)
Lithium heparin(separator tube)	63	34	3646	1.00	1.00 (0.99,1.01)	-1.91 (-2.59, -0.85)
Serum (separator tube)	63	36	3591	1.00	1.00 (1.00, 1.01)	0.30 (-0.44,0.77)
Sodium heparin	63	35	3595	1.00	0.99 (0.99, 1.00)	0.48 (-0.07, 1.08)

*Min, Max from the Evaluation Tube Type

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable

2. Clinical Specificity:

   Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

The expected values for alkaline phosphatase in serum are from literature references.

Age	Range (U/L)
*29 days to < 1 year	134 – 518
1 year to < 3 years	156 – 369
3 to 5 years	144 – 327
6 to 10 years	153 – 367
11 to 15 years, Male	113 – 438
11 to 15 years, Female	64 – 359
16 to 21 years, Male	56 – 167
16 to 21 years, Female	44 – 107
22 to 79 years, Female	50 –116
30 to 79 years, Female	46 –122

*This age interval has been modified to exclude neonates.

Rifai N, Horvath AR, Wittwer C, editors, Tietz Textbook of Clinical Chemistry and Molecular Molecular Diagnostics, 6th ed. St. Louis, Mo: Elsevier: 2018.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.