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

A. 510(k) Number:
k113374

B. Purpose for Submission:
New device

C. Measurand:
Albumin
Total Protein
Calcium
Phosphorus

D. Type of Test:
Quantitative photometric test

E. Applicant:
Alfa Wassermann Diagnostic Technologies, LLC

F. Proprietary and Established Names:
ACE Albumin Reagent
ACE Total Protein Reagent
ACE Calcium-Arsenazo Reagent
ACE Inorganic Phosphorus U.V. Reagent

G. Regulatory Information:

1. Regulation section:
   21 CFR§ 862.1035, Albumin test system
   21 CFR§ 862.1635, Total protein test system
   21 CFR§ 862.1145, Calcium test system
   21 CFR§ 862.1580, Phosphorus (inorganic) test system
2. Classification:
   Class II: Albumin test system and Calcium test system
   Class II, for Total protein test system, meets limits of exemptions per 21 CFR § 862.9 (c)(9)
   Class I, reserved for Phosphorus (inorganic) test system

3. Product code:
   CIX, bromcresol green dye-binding, albumin
   CEK, biuret (colorimetric), total protein
   CJY, azo dye, calcium
   CEO, phosphomolybdate (colorimetric), inorganic phosphorus

4. Panel:
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):
   The ACE Albumin Reagent is intended for the quantitative determination of albumin concentration in serum using the ACE Axcel Clinical Chemistry System. Albumin measurements are used in the diagnosis and treatment of numerous diseases involving primarily the liver or kidneys. This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.

   The ACE Total Protein Reagent is intended for the quantitative determination of total protein concentration in serum using the ACE Axcel Clinical Chemistry System. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic or nutritional disorders. This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.

   The ACE Calcium-Arsenazo Reagent is intended for the quantitative determination of calcium concentration in serum using the ACE Axcel Clinical Chemistry System. Calcium measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms). This test is intended for use in clinical laboratories or physician office laboratories. For *in vitro* diagnostic use only.

   The ACE Inorganic Phosphorus U.V. Reagent is intended for the quantitative determination of inorganic phosphorus concentration in serum using the ACE Axcel Clinical Chemistry System. Measurements of inorganic phosphorus are used in the diagnosis and treatment of various disorders, including parathyroid gland and kidney diseases and vitamin D imbalance. 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 *in vitro* diagnostic use only. For prescription and point-of-care use.

4. Special instrument requirements:
   ACE Axcel Clinical Chemistry System

I. Device Description:

The ACE Albumin Reagent consists of a single reagent bottle containing 0.39 mmol/L Bromcresol Green, acetate buffer, preservative and surfactant.

The ACE Total Protein Reagent consists of a single reagent bottle containing 12 mmol/L copper sulfate, 32mmol/L sodium potassium tartrate, 30 mmol/L potassium iodide, 600 mmol/L sodium hydroxide and non-reactive ingredients.

The ACE Calcium-Arsenazo Reagent consists of a single reagent bottle containing ≥ 0.15 mmol/L Arsenazo III, buffer and surfactant.

The ACE Inorganic Phosphorus U.V. Reagent consists of a single reagent bottle containing 0.48 mmol/L ammonium molybdate, 220 mmol/L sulfuric acid and surfactant.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Alfa Wassermann ACE plus ISE Clinical Chemistry System

2. Predicate K number(s):
   k930104

3. Comparison with predicate:

Item	Candidate Device	Predicate Device
Similarities and Differences for Albumin		
Intended Use	Intended for the quantitative determination of albumin concentration in serum. Albumin measurements are used in the diagnosis and treatment of numerous diseases involving primarily the liver or kidneys.	Same
Instrument	ACE Axcel Clinical Chemistry System	ACE Clinical Chemistry System
Calibration	GEMCAL calibrators	Same
Calibration Stability	30 days	Same
Basic Principle	Fiske/Subbarow method	Same
Analysis Temperature	37ºC	Same
Reaction Type	Endpoint	Same
Sample Type	Serum	Same
Expected Values	3.5 to 5.0 g/dL	Same
Reportable Range	0.3 to 7.0 g/dL	0.1 to 7.0 g/dL

Item	Candidate Device	Predicate Device
Similarities and Differences for Total Protein		
Intended Use	Intended for the quantitative determination of total protein concentration in serum. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic or nutritional disorders.	Same
Instrument	ACE Axcel Clinical Chemistry System	ACE Clinical Chemistry System
Calibration	GEMCAL calibrators	Same
Calibration Stability	30 days	Same
Basic Principle	Chemical method for total protein	Same
Analysis Temperature	37ºC	Same
Reaction Type	Endpoint	Same
Sample Type	Serum	Same
Expected Values	6.0 to 8.3 g/dL	Same
Reportable Range	0.4 to 14.0 g/dL	0.0 to 14.0 g/dL

Item	Candidate Device	Predicate Device
Similarities and Differences for Calcium		
Intended Use	Intended for the quantitative determination of calcium concentration in serum. Calcium measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease and tetany (intermittent muscular contractions or spasms).	Same
Instrument	ACE Axcel Clinical Chemistry System	ACE Clinical Chemistry System
Calibration	GEMCAL calibrators	Same
Calibration Stability	30 days	Same
Basic Principle	Chemical method for calcium	Same
Analysis Temperature	37ºC	Same
Reaction Type	Endpoint	Same
Sample Type	Serum	Same
Expected Values	8.5 to 10.5 mg/dL	Same
Reportable Range	0.4 to 15 mg/dL	0.3 to 15.0 mg/dL

Item	Candidate Device	Predicate Device
Similarities and Differences for Phosphorus		
Intended Use	Intended for the quantitative determination of inorganic phosphorus concentration in serum. Measurements of inorganic phosphorus are used in the diagnosis and treatment of various disorders, including parathyroid gland and kidney diseases and vitamin D imbalance.	Same
Instrument	ACE Axcel Clinical Chemistry System	ACE Clinical Chemistry System
Calibration	GEMCAL calibrators	Same
Calibration Stability	20 days	Same
Basic Principle	Phosphomolybdate method	Same
Analysis Temperature	37ºC	Same
Reaction Type	Endpoint	Same
Sample Type	Serum	Same
Expected Values	2.7 to 4.5 mg/dL	Same
Reportable Range	0.3 to 20 mg/dL	0.2 to 20.0 mg/dL

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

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

L. Test Principle:

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision of the Albumin, Total Protein, Calcium and Phosphorus tests on the ACE Axcel Clinical Chemistry System was evaluated in accordance with CLSI EP5-A2 in-house. Four levels were assayed 2 times per run, 2 runs per day, for a total of 22 days on 1 analyzer for a total of 88 measurements (n) per level. A set of three serum based pools (samples 1-3) and one normal human

serum pool (sample 4) were used. The results are summarized below:

Albumin:

Sample	Mean (g/dL)	Within run		Between run		Between day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	2.30	0.04	1.7%	0.0	0.0%	0.02	1.0%	0.05	2.0%
2	4.05	0.04	0.9%	0.03	0.6%	0.03	0.9%	0.06	1.4%
3	5.63	0.05	1.0%	0.01	0.2%	0.04	0.7%	0.07	1.2%
4	4.34	0.05	1.1%	0.01	0.2%	0.03	0.7%	0.06	1.4%

Total Protein:

Sample	Mean (g/dL)	Within run		Between run		Between day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	3.46	0.08	2.4%	0.04	1.2%	0.04	1.1%	0.10	2.9%
2	6.86	0.11	1.6%	0.00	0.0%	0.07	1.0%	0.13	1.9%
3	10.01	0.08	0.8%	0.05	0.5%	0.01	0.1%	0.10	1.0%
4	6.58	0.10	1.4%	0.05	0.7%	0.01	0.2%	0.11	1.6%

Calcium:

Sample	Mean (mg/dL)	Within run		Between run		Between day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	6.52	0.15	2.3%	0.00	0.0%	0.00	0.0%	0.15	2.3%
2	9.71	0.13	1.4%	0.00	0.0%	0.03	0.3%	0.13	1.4%
3	12.46	0.19	1.5%	0.06	0.4%	0.06	0.5%	0.21	1.7%
4	8.99	0.12	1.3%	0.16	1.7%	0.00	0.0%	0.20	2.2%

Phosphorus:

Sample	Mean (mg/dL)	Within run		Between run		Between day		Total	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	3.75	0.06	1.6%	0.02	0.5%	0.02	0.5%	0.07	1.7%
2	8.24	0.12	1.4%	0.00	0.0%	0.05	0.6%	0.13	1.5%
3	12.60	0.24	1.9%	0.00	0.0%	0.05	0.4%	0.24	1.9%
4	4.51	0.08	1.7%	0.08	1.8%	0.00	0.0%	0.11	2.5%

Additional precision studies were performed at 3 physical office lab (POL) sites. Studies consisted of running 3 serum based pools on 3 analyzers (one at each POL site) by one POL operator at site 1 and 2 and 2 POL operators at site 3, at least 3 times per run, 1 run per day, for a total of 5 days for a total n of 15 for each level at each site. The results are summarized below:

Albumin:

Lab	Sample	Mean (g/dL)	Within run		Total	
			SD	CV%	SD	CV%
POL 1	1	2.30	0.00	0.0%	0.0	0.0%
POL 2	1	2.36	0.00	0.0%	0.05	2.3%
POL 3	1	2.29	0.04	1.6%	0.04	1.6%
POL 1	2	4.01	0.06	1.6%	0.06	1.6%
POL 2	2	4.12	0.05	1.2%	0.08	2.0%
POL 3	2	4.01	0.05	1.2%	0.06	1.5%
POL 1	3	5.61	0.06	1.2%	0.06	1.2%
POL 2	3	5.73	0.04	0.7%	0.09	1.6%
POL 3	3	5.57	0.04	0.7%	0.10	1.9%

Total Protein:

Lab	Sample	Mean (g/dL)	Within run		Total	
			SD	CV%	SD	CV%
POL 1	1	3.49	0.04	1.1%	0.05	1.5%
POL 2	1	3.63	0.04	1.1%	0.05	1.4%
POL 3	1	3.52	0.04	1.1%	0.06	1.6%
POL 1	2	6.89	0.05	0.7%	0.06	0.9%
POL 2	2	7.13	0.09	1.3%	0.11	1.6%
POL 3	2	6.97	0.06	0.9%	0.11	1.6%
POL 1	3	10.17	0.08	0.8%	0.08	0.8%
POL 2	3	10.51	0.11	1.0%	0.12	1.1%
POL 3	3	10.27	0.11	1.0%	0.11	1.0%

Calcium:

Lab	Sample	Mean (mg/dL)	Within run		Total	
			SD	CV%	SD	CV%
POL 1	1	6.50	0.06	0.9%	0.08	1.2%
POL 2	1	6.43	0.09	1.4%	0.11	1.8%
POL 3	1	6.46	0.06	0.9%	0.19	2.9%
POL 1	2	9.68	0.10	1.0%	0.13	1.3%
POL 2	2	9.65	0.08	0.8%	0.11	1.1%
POL 3	2	9.69	0.13	1.4%	0.13	1.4%
POL 1	3	12.43	0.16	1.3%	0.20	1.6%
POL 2	3	12.33	0.15	1.2%	0.15	1.2%
POL 3	3	12.55	0.12	1.0%	0.14	1.1%

Phosphorus:

Lab	Sample	Mean (mg/dL)	Within run		Total	
			SD	CV%	SD	CV%
POL 1	1	3.65	0.06	1.6%	0.10	2.6%
POL 2	1	3.95	0.10	2.6%	0.10	2.6%
POL 3	1	3.77	0.12	3.2%	0.15	3.9%
POL 1	2	8.03	0.10	1.2%	0.12	1.5%
POL 2	2	8.53	0.11	1.3%	0.11	1.3%
POL 3	2	8.15	0.05	0.6%	0.08	1.0%
POL 1	3	12.41	0.13	1.0%	0.17	1.4%
POL 2	3	13.12	0.27	2.0%	0.27	2.0%
POL 3	3	12.69	0.08	0.6%	0.13	1.0%

b. *Linearity/assay reportable range:*

The linearity of the Albumin, Total Protein, Calcium and Phosphorus tests on the ACE Axcel Clinical Chemistry System was evaluated in accordance with CLSI EP6-A. Serum samples were spiked and dilutions of the spiked serum were then prepared by mixing the spiked serum in known proportions with saline (for albumin, total protein and phosphorus or diluted serum for calcium. Twelve levels were prepared for each analyte and each level was run in triplicate on one analyzer. The mean value of each set of triplicate determinations was calculated and the % recovery was calculated for each sample. The execution of first, second and third order linear regression did not indicate statistically significant non linear regression coefficients in either the 2nd order or 3rd order models. Linear regression analysis of the assigned values (x axis) versus the measured values (y axis) was also evaluated. The results of these studies are summarized below:

Albumin:
Linear regression analysis demonstrated that the assay is linear from 0.1 to 7.1 g/dL ($y = 0.997x + 0.12$; $r^2 = 0.9979$). The sponsor claims the reportable range for the assay is from 0.3 to 7.0 g/dL.

Total Protein:
Linear regression analysis demonstrated that the assay was linear from 0.37 to 15.4 g/dL ($y = 1.015x + 0.01$; $r^2 = 0.9984$). The sponsor claims that the reportable range for the assay is from 0.4 to 14 g/dL.

Calcium:
Linear regression analysis demonstrated that the assay was linear from 0.37 to 15.9 mg/dL ($y = 1.019x - 0.11$; $r^2 = 0.9994$). The sponsor claims that the reportable range of the assay is from 0.4 to 15 mg/dL.

Inorganic Phosphorus:

Linear regression analysis demonstrated that the assay was linear from 0.3 to 20.0 mg/dL ($y = 1.016x – 0.10$; $r^2 = 0.9890$). The sponsor claims that the reportable range of the assay is from 0.3 to 20 mg/dL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The GEMCAL calibrators recommended for use with these reagents were cleared under k930104.

Alfa Wassermann Level 1 and level 2 Chemistry Controls recommended for use with these reagents were cleared under k930104.

d. *Detection limit*:
The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) 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^{th} and 58^{th} 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:

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

The LoQ was determined using 40 replicates of 5 low level specimens and defined as the lowest concentration for which the CV is less than 20%.

The estimate for LoB is 0.07 g/dL (Albumin), 0.10 g/dL (Total Protein), 0.05 mg/dL (Calcium) and 0.04 mg/dL (Phosphorus)

The estimate for LoD is 0.09 g/dL (Albumin), 0.15 g/dL (Total Protein), 0.11 mg/dL (Calcium) and 0.07 mg/dL (Phosphorus).

The estimate for LoQ is 0.09 g/dL (Albumin), 0.31 g/dL (Total Protein), 0.20 mg/dL (Calcium) and 0.22 mg/dL (Phosphorus).

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 and abnormal 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 ACE Axcel Clinical Chemitry System. The following interference studies were performed:

Interferent	Concentrations tested (mg/dL)	
	Low	High
Ascorbic acid	0.188	6
Unconjugated bilirubin	1.56	50
Hemoglobin	31.3	1000
Intralipid (lipemia)	62.5	2000

Analyte	Analyte concentration	
	Low pool	High pool
Albumin	3.0 – 4.0 g/dL	5.2 – 5.7 g/dL
Total Protein	5.8 – 6.6 g/dL	8.5 – 11.9 g/dL
Calcium	7.6 – 8.5 mg/dL	11.0 – 13.5 mg/dL
Phosphorus	2.9 – 4.3 mg/dL	8.9 – 13.7 mg/dL

The sponsor states that interferences are considered to be non-significant if the bias between the tested and control samples are within ±10% for all the analytes. The tested ranges, analyte concentrations and interference claims are listed in the tables below:

Interferent	**Assay**			
	Albumin	Total	Calcium	Phosphorus
Unconjugated bilirubin	58 mg/dL	30 mg/dL	55 mg/dL	8 mg/dL
Hemolysis	250 mg/dL	250 mg/dL	1000 mg/dL	31.3 mg/dL
Lipemia (Intralipid)	2000 mg/dL	615 mg/dL	1000 mg/dL	554 mg/dL
Ascorbic Acid	6 mg/dL	6 mg/dL	6 mg/dL	6 mg/dL

Other limitations:

- The product insert for the Inorganic Phosphorus Reagent includes a limitation that hemolyzed, icteric or lipemic samples should not be used.
- Each product insert states that clear unhemolyzed serum should be used.
- Each product insert refers to literature for a comprehensive list of drugs and other substances that can affect the analyte concentrations in serum.

f. Assay cut-off:
Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:
In-House Studies:
Serum samples were evaluated in singlicate on the ACE Axcel Clinical Chemistry System. For comparison, the same samples were evaluated in singlicate using the predicate device. Diluted and spiked samples were

included in the studies (no more than 10% of total samples). 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:

Albumin

n	Range (g/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95% CI Intercept
118	0.4 to 6.4	y = 0.996x + 0.03	0.9959	0.980 to 1.013	-0.04 to 0.10

Total Protein

n	Range (g/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95% CI Intercept
121	0.4 to 13.5	y = 0.990x – 0.03	0.9977	0.978 to 1.002	-0.12 to 0.06

Calcium

n	Range (mg/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95% CI Intercept
111	0.7 to 14.5	y = 1.020x – 0.29	0.9935	0.998 to 1.042	-0.50 to -0.08

Phosphorus

n	Range (mg/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95% CI Intercept
110	0.6 to 19.6	y = 1.005x – 0.01	0.9983	0.994 to 1.017	-0.06 to 0.05

POL studies:
Serum samples were evaluated in singlicate on the ACE Axcel Clinical Chemistry System at 3 POLs. For comparison, the same samples were evaluated in singlicate using the predicate device. Diluted and spiked samples were included in the studies. One operator performed the studies at sites 1 and 2 and 2 operators performed the study at site 3. 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:

Albumin:

Lab	n	Range (g/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95%CI Intercept
1	60	1.2-6.1	y = 0.992x + 0.07	0.9944	0.964 to 1.019	-0.05 to 0.19
2	57	0.5-6.3	y = 1.014x - 0.04	0.9966	0.991 to 1.037	-0.14 to 0.06
3	45	1.1-6.7	y = 0.991x + 0.20	0.9894	0.946 to 1.035	0.02 to 0.39

Total Protein:

Lab	n	Range (g/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95%CI Intercept
1	60	1.4-13.2	y = 0.986x + 0.01	0.9987	0.973 to 0.999	-0.09 to 0.10
2	58	0.7-12.6	y = 1.022x + 0.02	0.9966	1.000 to 1.045	-0.14 to 0.19
3	45	1.5-13.7	y = 1.011x - 0.14	0.9932	0.974 to 1.047	-0.41 to 0.13

Calcium:

Lab	n	Range (mg/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95%CI Intercept
1	62	1.6-14.0	y = 1.003x - 0.02	0.9977	0.985 to 1.021	-0.19 to 0.14
2	59	0.6-13.6	y = 0.991x - 0.21	0.9965	0.969 to 1.013	-0.42 to 0.00
3	46	3.1-14.0	y = 1.030x + 0.00	0.9895	0.985 to 1.075	-0.43 to 0.42

Inorganic Phosphorus:

Lab	n	Range (mg/dL)	Regression Equation	Correlation Coefficient	95% CI Slope	95%CI Intercept
1	52	0.9-18.9	y = 1.047x - 0.24	0.9977	1.027 to 1.067	-0.33 to -0.15
2	48	0.3-18.9	y = 1.031x + 0.01	0.9996	1.022 to 1.039	-0.05 to 0.06
3	48	1.3-19.8	y = 1.028x + 0.01	0.9990	1.014 to 1.041	-0.06 to 0.09

b. Matrix comparison:
The device is being cleared for serum use only.

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:

The sponsor cites the following expected values from literature:

Analyte	Expected values
Albumin[2]	3.5 to 5 g/dL
Total Protein[2]	6.4 to 8.3 g/dL (ambulatory)
	6.0 to 7.8 g/dL (recumbent)
Calcium[1]	8.5 to 10.2 mg/dL
Inorganic Phosphorus[2]	2.7 to 4.5 mg/dL

[1]Burtis, C.A., Ashwood, E.R. (Eds.), Tietz Fundamentals of Clinical Chemistry, 4th Edition, W.B. Saunders Co., Philadelphia, PA (1996).
[2]Tietz, N.W. (Ed.), Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Sanders Co., Philadelphia, PA (1995).

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.