

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

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

k113435

**B. Purpose for Submission:**

New device

**C. Measurand:**

Carbon Dioxide, Direct Bilirubin, Total Bilirubin, and Magnesium

**D. Type of Test:**

Quantitative

Enzymatic activity (CO<sub>2</sub>), Diazo Colorimetry (Direct and Total Bilirubin), and Photometric (Magnesium)

**E. Applicant:**

Alfa Wassermann

**F. Proprietary and Established Names:**

ACE Carbon Dioxide (CO<sub>2</sub>-LC) Reagent  
ACE Direct Bilirubin Reagent  
ACE Total Bilirubin Reagent  
ACE Magnesium Reagent

**G. Regulatory Information:**

<b>Product Code</b>	<b>Classification</b>	<b>Regulation Section</b>	<b>Panel</b>
KHS	II	862.1160, Bicarbonate/Carbon-Dioxide test system	75-Chemistry
CIG	II	862.1110, Bilirubin (total or direct) test system	75-Chemistry
JGJ	II	862.1495, Magnesium test system	75-Chemistry

## H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The ACE Carbon Dioxide (CO<sub>2</sub>-LC) Reagent is intended for the quantitative determination of carbon dioxide in serum using the ACE Axcel Clinical Chemistry System. Bicarbonate/carbon dioxide measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with changes in body acid-base balance. This test is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

The ACE Direct Bilirubin Reagent is intended for the quantitative determination of direct bilirubin in serum using the ACE Axcel Clinical Chemistry Systems. Measurements of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological and metabolic disorders, including hepatitis and gall bladder block. This test is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

The ACE Total Bilirubin Reagent is intended for the quantitative determination of total bilirubin in serum using the ACE Axcel Clinical Chemistry System. Measurements of the levels of bilirubin, an organic compound formed during the normal and abnormal destruction of red blood cells, is used in the diagnosis and treatment of liver, hemolytic, hematological and metabolic disorders, including hepatitis and gall bladder block. This test is intended for use in clinical laboratories or physician office laboratories. For in vitro diagnostic use only.

The ACE Magnesium Reagent is intended for the quantitative determination of magnesium in serum using the ACE Axcel Clinical Chemistry System. Magnesium measurements are used in the diagnosis and treatment of hypomagnesemia (abnormally low plasma levels of magnesium) and hypermagnesemia (abnormally high plasma levels of magnesium). 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 *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 Carbon Dioxide Reagent comes in a kit containing 4 x 7.5 mL Carbon Dioxide Reagent and 1 x 15 mL 30 mEq/L Carbon Dioxide Standard. The kit supports 500 tests.

The ingredients of the reagents are:

#### **Carbon Dioxide Reagent**

Phosphoenolpyruvate (PEP) 63 mmol/L

Nicotinamide adenine dinucleotide (NADH) analog, reduced 3.0 mmol/L

Phosphoenol pyruvate carboxylase (PEPC) (Microbial) >2000 U/L

Malate dehydrogenase (MD) (Mammalian) >20 KU/L

Buffer (pH 7.5 @ 25°C)

Activators, Stabilizers, Surfactant and Preservative

#### **Carbon Dioxide Standard**

Sodium carbonate 30 mEq/L, Buffer and Preservative

The ACE Direct Bilirubin Reagent comes in a kit containing 3x12 mL Direct Bilirubin Reagent and 3x3mL Sodium Nitrite Reagent. Each kit supports 120 tests.

The ingredients of the reagents are:

#### **Direct Bilirubin Reagent**

Sulfanilic acid 35.6 mmol/L

Hydrochloric acid 165 mmol/L

#### **Sodium Nitrite Reagent**

Sodium nitrite 43.5 mmol/L

The ACE Total Bilirubin Reagent comes in a kit containing 3x30 mL Total Bilirubin Reagent and 3x6mL Sodium Nitrite Reagent. Each kit supports 300 tests.

The ingredients of the reagents are:

#### **Total Bilirubin Reagent**

Sulfanilic acid 35.6 mmol/L

Hydrochloric acid 165 mmol/L

Dimethyl sulfoxide (DMSO) 50% (v/v)

#### **Sodium Nitrite Reagent**

Sodium nitrite 60.0 mmol/L

The ACE Magnesium Reagent comes in a kit containing 6x12 mL liquid ready-to-use bottles. Each kit supports 160 tests.

ACE Magnesium Reagent has the following composition:  
 Xylidyl blue-1 0.14 mmol/L  
 EGTA 0.1 mmol/L  
 Buffer and Surfactant

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

ACE Clinical Chemistry System, ACE Carbon Dioxide (CO<sub>2</sub>-LC) Reagent  
 ACE Clinical Chemistry System, ACE Direct Bilirubin Reagent  
 ACE Clinical Chemistry System, ACE Total Bilirubin Reagent  
 ACE Clinical Chemistry System, ACE Magnesium Reagent

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

k931786

3. Comparison to predicate

Items	ACE Axcel Clinical Chemistry System, ACE Carbon Dioxide (CO <sub>2</sub> -LC) Reagent ( <b>Candidate Device</b> )	ACE Clinical Chemistry System, ACE Carbon Dioxide (CO <sub>2</sub> -LC) Reagent ( <b>Predicate Device</b> )
<b>Similarity</b>		
Intended use /Indication for use	Same	For the quantitative determination of carbon dioxide in serum. Bicarbonate/carbon dioxide measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with changes in body acid-base balance. For in vitro diagnostic use only.
Test Principle	Same	Phosphoenolpyruvate (PEP) and HCO <sub>3</sub> <sup>-</sup> react to form oxaloacetate and phosphate in the presence of phosphoenolpyruvate carboxylase. Malate dehydrogenase catalyzes the reaction of oxaloacetate and reduced nicotinamide adenine dinucleotide (NADH) to NAD <sup>+</sup> and malate. The change in absorbance due to the conversion

		of NADH to NAD <sup>+</sup> is directly proportional to the amount of CO <sub>2</sub> in the sample.
Reaction Type	Same	Kinetic
Reactive Ingredients	Same	Phosphoenolpyruvate NADH Phosphoenol pyruvate decarboxylase Malate dehydrogenase Buffer (pH 7.5 at 25°C)
Non-reactive Ingredients	Same	Activators, stabilizers, surfactant and preservative
Sample Type	Same	Serum
Sample Volume	Same	6 µL
Reaction Volume (total)	Same	156 µL
Calibration	Same	30 mEq/L Carbon Dioxide Standard
<b>Difference</b>		
Instrument Platforms	ACE Axcel Clinical Chemistry System	ACE and ACE <i>Alera</i> <sup>®</sup> Clinical Chemistry Systems
Detection Limit	1.2 mEq/L	2 mEq/L
Reportable Range	4 to 50 mEq/L	2 to 50 mEq/L

<b>Items</b>	<b>ACE Axcel Clinical Chemistry System, ACE Direct Bilirubin Reagent (Candidate Device)</b>	<b>ACE Clinical Chemistry System, ACE Direct Bilirubin Reagent (Predicate Device)</b>
<b>Similarity</b>		
Intended use /Indication for use	Same	For the quantitative determination of direct bilirubin in serum. For in vitro diagnostic use only.
Test Principle	Same	Reaction of direct bilirubin with diazotized sulfanilic acid to form azobilirubin; resulting increase in absorbance measured, one minute after sample addition, bichromatically at 554/692 nm.
Reaction	Same	Endpoint

Type		
Reactive Ingredients	Same	Sulfanilic acid Hydrochloric acid Sodium nitrite
Non-reactive Ingredients	Same	None
Sample Type	Same	Serum
Sample Volume	Same	20 µL
Reaction Volume (total)	Same	355 µL
Reportable Range	Same	2 to 50 mEq/L
Calibration	Same	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the calibrator. The use of GEMCAL Reference Serum is recommended.
<b>Difference</b>		
Instrument Platforms	ACE Axcel Clinical Chemistry System	ACE and ACE <i>Alera</i> <sup>®</sup> Clinical Chemistry Systems
Detection Limit	0.1 mg/dL	0 mg/dL
Reportable Range	0.1 to 14.0 mg/dL	Up to 14.0 mg/dL

<b>Items</b>	<b>ACE Axcel Clinical Chemistry System, ACE Total Bilirubin Reagent (Candidate Device)</b>	<b>ACE Clinical Chemistry System, ACE Total Bilirubin Reagent (Predicate Device)</b>
<b>Similarity</b>		
Intended use /Indication for use	Same	For the quantitative determination of total bilirubin in serum. For in vitro diagnostic use only.
Test Principle	Same	Reaction of total bilirubin with diazotized sulfanilic acid to form azobilirubin; resulting increase in absorbance measured, one minute after sample addition, bichromatically at 554/692 nm.
Reaction Type	Same	Endpoint

Reactive Ingredients	Same	Sulfanilic acid Hydrochloric acid Dimethyl sulfoxide (DMSO) Sodium nitrite
Non-reactive Ingredients	Same	None
Sample Type	Same	Serum
Sample Volume	Same	20 µL
Reaction Volume (total)	Same	380 µL
Calibration	Same	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the calibrator. The use of GEMCAL Reference Serum is recommended.
<b>Difference</b>		
Instrument Platforms	ACE Axcel Clinical Chemistry System	ACE and ACE <i>Alera</i> <sup>®</sup> Clinical Chemistry Systems
Detection Limit	0.1 mg/dL	0 mg/dL
Reportable Range	0.2 to 40.0 mg/dL	Up to 40.0 mg/dL

<b>Items</b>	ACE Axcel Clinical Chemistry System, ACE Magnesium Reagent <b>(Candidate Device)</b>	ACE Clinical Chemistry System, ACE Magnesium Reagent <b>(Predicate Device)</b>
<b>Similarity</b>		
Intended use /Indication for use	Same	For the quantitative determination of Magnesium in serum. For in vitro diagnostic use only.
Test Principle	Same	Magnesium ions in serum react with Xylidyl blue-1 in an alkaline medium to produce a red complex which is measured bichromatically at 525 nm/692 nm. The intensity of the color produced is directly proportional to the magnesium concentration. EGTA prevents calcium interference by preferential chelation of calcium

		present in the sample. A surfactant system is included to remove protein interference.
Reaction Type	Same	Endpoint
Reactive Ingredients	Same	Xylidyl blue-1 EGTA
Non-reactive Ingredients	Same	Buffer Surfactant
Sample Type	Same	Serum
Sample Volume	Same	3 $\mu$ L
Reaction Volume (total)	Same	488 $\mu$ L
Calibration	Same	Calibrated by referencing the change in absorbance of the unknown samples to the change in absorbance of the calibrator. The use of GEMCAL Reference Serum is recommended.
<b>Difference</b>		
Instrument Platforms	ACE Axcel Clinical Chemistry System	ACE and ACE <i>Alera</i> <sup>®</sup> Clinical Chemistry Systems
Detection Limit	0.1 mg/dL	0 mg/dL
Reportable Range	0.4 to 6.0 mg/dL	Up to 6.0 mg/dL

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition  
 CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures, A Statistical Approach; Approved Guideline  
 CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition  
 CLSI EP9-A2-IR: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition  
 CLSI EP10-A3: Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures; Approved Guideline-Third Edition  
 CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

**L. Test Principle:**

In the ACE CO2 Assay, carbon dioxide (in the form of bicarbonate HCO3-) reacts with phosphoenolpyruvate (PEP) in the presence of phosphoenolpyruvate carboxylase (PEPC) and magnesium to yield oxaloacetic acid (OAA) and phosphate. In the presence of malate dehydrogenase (MD), the reduced cofactor is oxidized by oxaloacetic acid. The reduced cofactor absorbs strongly at 408 nm whereas its oxidized form does not. The rate of decrease in absorbance, monitored bichromatically at 408 nm/692 nm, is proportional to the carbon dioxide content of the sample.

In the ACE Direct Bilirubin Assay, sodium nitrite added to sulfanilic acid forms diazotized sulfanilic acid. Bilirubin glucuronide in serum reacts with diazotized sulfanilic acid to form azobilirubin, which absorbs strongly at 554 nm. The increase in absorbance, measured bichromatically at 554 nm/692 nm, one minute after sample addition, is directly proportional to the direct bilirubin concentration.

In the ACE Total Bilirubin Assay, sodium nitrite, when added to sulfanilic acid, forms diazotized sulfanilic acid. Bilirubin in serum reacts with diazotized sulfanilic acid to form azobilirubin, which absorbs strongly at 554 nm. The inclusion of DMSO in the reagent as an accelerator, causes both direct and indirect bilirubin to react rapidly. The increase in absorbance, measured bichromatically at 554 nm/692 nm, is directly proportional to the total bilirubin concentration.

In the ACE Magnesium Assay, magnesium ions in serum react with Xylidyl blue-1 in an alkaline medium to produce a red complex which is measured bichromatically at 525 nm/692 nm. The intensity of color produced is directly proportional to the magnesium concentration. EGTA prevents calcium interference by preferential chelation of calcium present in the sample. A surfactant system is included to remove protein interference.

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

1. Analytical performance:

a. *Precision/Reproducibility:*

In-house precision

Precision studies were conducted by testing human serum pools at four levels. The samples were run 2 times per run, 2 runs per day, for a total of 22 days using one instrument. Results are summarized below.

CO2:

Sample	Mean (mEq/L)	Within Run	Between Run	Between Day	Total
1	14.25	SD 0.27	SD 0.34	SD 0.35	SD 0.55
		CV 1.9%	CV 2.4%	CV 2.4%	CV 3.9%
2	21.68	SD 1.49	SD 0.56	SD 0.00	SD 1.59
		CV 6.9%	CV 2.6%	CV 0.0%	CV 7.4%

3	32.62	SD 1.88	SD 0.63	SD 0.00	SD 1.98
		CV 5.8%	CV 1.9%	CV 0.0%	CV 6.1%
4	22.53	SD 0.35	SD 0.63	SD 0.64	SD 0.97
		CV 1.6%	CV 2.8%	CV 2.8%	CV 4.3%

**Direct Bilirubin:**

Sample	Mean (mg/dL)	Within Run	Between Run	Between Day	Total
1	0.30	SD 0.02	SD 0.00	SD 0.00	SD 0.02
		CV 7.2%	CV 0.0%	CV 0.0%	CV 7.2%
2	5.16	SD 0.07	SD 0.04	SD 0.06	SD 0.10
		CV 1.4%	CV 0.7%	CV 1.1%	CV 1.9%
3	9.03	SD 0.08	SD 0.00	SD 0.12	SD 0.14
		CV 0.8%	CV 0.0%	CV 1.3%	CV 1.5%
4	0.21	SD 0.04	SD 0.00	SD 0.00	SD 0.04
		CV 16.5%	CV 0.0%	CV 2.3%	CV 16.6%

**Total Bilirubin:**

Sample	Mean (mg/dL)	Within Run	Between Run	Between Day	Total
1	0.58	SD 0.04	SD 0.02	SD 0.00	SD 0.05
		CV 7.3%	CV 4.1%	CV 0.0%	CV 8.4%
2	13.05	SD 0.14	SD 0.00	SD 0.14	SD 0.19
		CV 1.0%	CV 0.0%	CV 1.1%	CV 1.5%
3	24.67	SD 0.14	SD 0.03	SD 0.24	SD 0.28
		CV 0.6%	CV 0.1%	CV 1.0%	CV 1.1%
4	0.53	SD 0.06	SD 0.00	SD 0.00	SD 0.06
		CV 10.6%	CV 0.0%	CV 0.0%	CV 10.6%

**Magnesium:**

Sample	Mean (mg/dL)	Within Run	Between Run	Between Day	Total
1	2.19	SD 0.12	SD 0.07	SD 0.03	SD 0.14
		CV 5.6%	CV 3.1%	CV 1.6%	CV 6.6%
2	3.89	SD 0.12	SD 0.03	SD 0.11	SD 0.17
		CV 3.1%	CV 0.9%	CV 2.8%	CV 4.2%
3*	4.83	SD 0.13	SD 0.08	SD 0.12	SD 0.20
		CV 2.7%	CV 1.6%	CV 2.6%	CV 4.1%
4	1.71	SD 0.10	SD 0.08	SD 0.00	SD 0.13
		CV 5.9%	CV 4.7%	CV 0.0%	CV 7.6%

\*Data collected for 21 days

Point-of-Care precision

Precision studies were also conducted at 3 Physician Office Laboratories (POL). Human serum pools and QC samples were tested in triplicate over at least 5 different days. The results are presented below:

CO<sub>2</sub>:

Lab	Sample	Mean (mEq/L)	%CV or SD (mg/dL)	
			Within-Run	Total
POL 1	1	16.89	SD 0.29	SD 0.69
			CV 1.7%	CV 4.1%
POL 2	1	16.16	SD 0.35	SD 0.85
			CV 2.2%	CV 5.3%
POL 3	1	18.41	SD 0.48	SD 1.04
			CV 2.6%	CV 5.7%

POL 1	2	27.58	SD 0.54	SD 0.79
			CV 2.0%	CV 2.9%
POL 2	2	27.40	SD 0.68	SD 1.33
			CV 2.5%	CV 4.9%
POL 3	2	29.58	SD 0.31	SD 0.53
			CV 1.0%	CV 1.8%

POL 1	3	38.64	SD 0.50	SD 0.91
			CV 1.3%	CV 2.3%
POL 2	3	37.34	SD 0.58	SD 1.34
			CV 1.6%	CV 3.6%
POL 3	3	39.66	SD 0.45	SD 0.88
			CV 1.1%	CV 2.2%

Direct Bilirubin:

Lab	Direct Bilirubin	Mean (mg/dL)	%CV or SD (mg/dL)	
			Within-Run	Total
POL 1	Control 1	0.9	SD 0.00	SD 0.00
			%CV 0.0%	%CV 0.0%
POL 2	Control 1	0.9	SD 0.03	SD 0.03
			%CV 3.3%	%CV 3.3%
POL 3	Control 1	0.9	SD 0.04	SD 0.04
			%CV 4.4%	%CV 4.4%
POL 1	Control 2	2.2	SD 0.04	SD 0.04
			%CV 1.8%	%CV 1.8%
POL 2	Control 2	2.3	SD 0.07	SD 0.07
			%CV 3.0%	%CV 3.0%
POL 3	Control 2	2.3	SD 0.08	SD 0.08
			%CV 3.5%	%CV 3.5%

POL 1	Sample 1	5.16	SD 0.06	SD 0.09
			%CV 1.2%	%CV 1.7%
POL 2	Sample 1	5.16	SD 0.11	SD 0.15
			%CV 2.1%	%CV 2.9%
POL 3	Sample 1	5.19	SD 0.10	SD 0.10
			%CV 2.0%	%CV 2.0%
POL 1	Sample 2	8.96	SD 0.13	SD 0.18
			%CV 1.5%	%CV 2.0%
POL 2	Sample 2	8.97	SD 0.09	SD 0.17
			%CV 1.0%	%CV 1.9%
POL 3	Sample 2	8.99	SD 0.08	SD 0.09
			%CV 0.9%	%CV 1.0%

Total Bilirubin:

Lab	Sample	Mean (mg/dL)	%CV or SD (mg/dL)	
			Within-Run	Total
POL 1	Control 1	1.5	SD 0.04	SD 0.04
			%CV 2.7%	%CV 2.7%
POL 2	Control 1	1.5	SD 0.05	SD 0.05
			%CV 3.3%	%CV 3.3%
POL 3	Control 1	1.5	SD 0.05	SD 0.05
			%CV 3.3%	%CV 3.3%
POL 1	Control 2	5.4	SD 0.00	SD 0.00
			%CV 0.0%	%CV 0.0%
POL 2	Control 2	5.4	SD 0.14	SD 0.14
			%CV 2.6%	%CV 2.6%
POL 3	Control 2	5.3	SD 0.08	SD 0.08
			%CV 1.5%	%CV 1.5%

POL 1	Sample 1	13.29	SD 0.19	SD 0.19
			%CV 1.5%	%CV 1.5%
POL 2	Sample 1	13.12	SD 0.21	SD 0.21
			%CV 1.6%	%CV 1.6%
POL 3	Sample 1	12.96	SD 0.17	SD 0.20
			%CV 1.3%	%CV 1.6%
POL 1	Sample 2	24.93	SD 0.23	SD 0.40
			%CV 0.9%	%CV 1.6%
POL 2	Sample 2	24.59	SD 0.48	SD 0.70
			%CV 2.0%	%CV 2.9%
POL 3	Sample 2	24.46	SD 0.26	SD 0.33
			%CV 1.1%	%CV 1.3%

**Magnesium:**

Lab	Sample	Mean (mg/dL)	%CV or SD (mg/dL)	
			Within-Run	Total
POL 1	1	2.22	SD 0.07	SD 0.07
			CV 3.1%	CV 3.1%
POL 2	1	2.22	SD 0.09	SD 0.10
			CV 4.1%	CV 4.6%
POL 3	1	2.06	SD 0.07	SD 0.14
			CV 3.4%	CV 6.9%
POL 1	2	4.02	SD 0.11	SD 0.12
			CV 2.7%	CV 2.9%
POL 2	2	4.07	SD 0.08	SD 0.11
			CV 2.0%	CV 2.6%
POL 3	2	3.80	SD 0.11	SD 0.15
			CV 2.9%	CV 3.9%
POL 1	3	5.69	SD 0.07	SD 0.11
			CV 1.2%	CV 2.0%
POL 2	3	5.73	SD 0.13	SD 0.13
			CV 2.4%	CV 2.4%
POL 3	3	5.38	SD 0.14	SD 0.20
			CV 2.6%	CV 3.8%

*b. Linearity/assay reportable range:*

Linearity across the assay range was confirmed by spiking serum samples to a high concentration of analyte, then diluting the sample to obtain 10 levels to cover the measuring range of each assay. The assigned value of the highest sample was set to its mean value. The assigned values of the other levels were calculated by multiplying the mean value by the dilution ratios. Each level was tested in replicates of 3. Results are presented below:

### CO2

Linear Regression:  $y = 0.998x + 1.3$ ,  $r^2 = 0.9958$

Based on the results of the linearity study and Limit of detection study (see below in *d*), the sponsor claimed that the assay's reportable range is 4-50 mEq/dL.

### Direct Bilirubin

Linear Regression:  $y = 0.990x - 0.113$ ,  $r^2 = 0.999$

Based on the results of the linearity study and Limit of detection study (see below in *d*), the sponsor claimed that the assay's reportable range is 0.1 to 14.0 mg/dL

### Total Bilirubin

Linear Regression:  $y = 1.003x + 0.18$ ,  $r^2 = 0.9998$

Based on the results of the linearity study and Limit of detection study (see below in *d*), the sponsor claimed that the assay's reportable range is 0.2 to 40.0 mg/dL

### Magnesium

Linear Regression:  $y = 0.987x + 0.07$ ,  $r^2 = 0.9983$

Based on the results of the linearity study and Limit of detection study (see below in *d*), the sponsor claimed that the assay's reportable range is 0.4 to 6.0 mg/dL

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

Traceability: CO2 method is traceable to NIST SRM 351. Total Bilirubin assay is traceable to NIST SRM 916. Direct Bilirubin assay is traceable to gravimetric standards prepared with NIST material. Magnesium method is traceable to NIST SRM 909.

The CO2 assay is calibrated by comparing the change in absorbance of the unknown sample to the change in absorbance of the 30 mEq/L CO2 standard included in the reagent kit. The CO2 standard was previously cleared under k854544. The Direct Bilirubin, Total Bilirubin and Magnesium assay is calibrated using the Gemcal Reference Serum previously cleared under k844344.

*d. Detection limit:*

The Limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) were determined according to CLSI EP17-A with the ACE Axcel Clinical Chemistry System. For the LoD studies, low samples and true blanks (n=60 reps, 20 reps per day) were tested over three days on two ACE Axcel Clinical Chemistry Systems. For the LoQ studies, samples (n = 40 reps, 8 reps per run) were tested in five separate runs over five days. The results are as follows:

Analyte	LoB	LoD	LoQ
CO2 (mEq/L)	1.0	1.2	2.9
Direct Bilirubin (mg/dL)	0.1	0.1	0.1
Total Bilirubin (mg/dL)	0.2	0.2	0.2
Mg (mg/dL)	0.2	0.2	0.4

e. *Analytical specificity:*

Interference studies were performed to determine the effects from potential interferents. The various concentration of interferent was spiked into serum pools containing analytes at normal and abnormal concentrations. All samples were tested in triplicate. Six interferent levels and the control were tested for each interferent. Interference is defined as a result that is different from the control by more than the least detectable dose of the assay (+/-0.1 mg/dL for direct and total bilirubin assay) or +/-10% for CO2 and Magnesium. The tested ranges and analyte concentrations are presented in the product labeling.

CO2:

Interferent Compound	Concentration with No Interference Up To
Ascorbic Acid	6 mg/dL
Unconjugated Bilirubin	55 mg/dL
Hemoglobin	500 mg/dL
Intralipid	1000 mg/dL

Direct Bilirubin:

Interferent Compound	Concentration with No Interference Up To
Ascorbic Acid	6 mg/dL
Hemoglobin	31.3 mg/dL*
Intralipid	542 mg/dL

\* Specimens showing indication of hemolysis should not be analyzed.

Total Bilirubin:

Interferent Compound	Concentration with No Interference Up To
Ascorbic Acid	6 mg/dL
Hemoglobin	62.5 mg/dL*
Intralipid	650 mg/dL

\*Specimens showing any indication of hemolysis should not be analyzed.

Magnesium:

Interferent Compound	Concentration with No Interference Up To
Ascorbic Acid	6 mg/dL
Unconjugated Bilirubin	30 mg/dL
Hemoglobin	125 mg/dL*
Intralipid	315 mg/dL

\*Specimens showing any indication of hemolysis should not be analyzed.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

An in-house method comparison study to the predicate device was performed with serum patient samples. A small number of the samples (<10%) were spiked or diluted to cover the low and high end of the assay range for each analyte. The results are presented in the table below:

Analyte	n	Regression Equation	r <sup>2</sup>	Sample range
CO <sub>2</sub>	119	y = 0.984x - 0.18	0.9735	6.4-47.6 mEq/L
Direct Bilirubin	116	y = 0.972x + 0.00	0.9997	0.2-12.5 mg/dL
Total Bilirubin	117	y = 0.966x + 0.01	0.9997	0.2-34.8 mg/dL
Magnesium	108	y = 0.998x + 0.03	0.9690	0.6-5.5 mg/dL

Additional method comparison studies were performed at three Physician Office Laboratories using patient serum specimens. The results are presented in the tables below:

CO <sub>2</sub>				
POL	n	Regression Equation	r <sup>2</sup>	Sample range (mEq/L)
1	56	y = 1.003x + 0.29	0.9819	6.5-43.6
2	52	y = 1.014x - 0.01	0.9917	8.2-49.0
3	46	y = 1.050x - 1.06	0.9952	5.6-49.4

Direct Bilirubin

POL	n	Regression Equation	r <sup>2</sup>	Sample range (mg/dL)
1	56	$y = 1.006x + 0.01$	0.9997	0.1-12.7
2	60	$y = 1.017x + 0.00$	0.9996	0.1-13.8
3	48	$y = 0.992x + 0.01$	0.9996	0.1-13.0

Total Bilirubin

POL	n	Regression Equation	r <sup>2</sup>	Sample range (mg/dL)
1	58	$y = 1.015x - 0.01$	1.0000	0.2-38.7
2	62	$y = 1.019x + 0.02$	0.9999	0.2-35.0
3	50	$y = 1.045x - 0.04$	0.9993	0.2-36.9

Magnesium

POL	n	Regression Equation	r <sup>2</sup>	Sample range (mg/dL)
1	49	$y = 0.957x + 0.06$	0.9917	0.7-5.7
2	47	$y = 0.986x + 0.13$	0.9930	0.9-5.9
3	47	$y = 1.037x - 0.25$	0.9858	0.6-5.6

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. Clinical studies are not typically submitted for this device type.

5. Expected values/Reference range:

The following expected values are provided in the product insert based on the literature for each analyte. The sponsor stated that each laboratory should determine the expected values for its particular population.

CO<sub>2</sub>: 23-29 mEq/L

Direct Bilirubin: <0.2 mg/dL

Total Bilirubin: 0.2-1.0 mg/dL

Mg: 1.3-2.2 mEq/L, 1.6-2.6 mg/dL

Tietz, N.W. (Ed.), Clinical Guide to Laboratory Tests, 4th Edition, W.B. Saunders Co., Philadelphia, PA (2006).

**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.