

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

A. 510(k) Number:

k140248

B. Purpose for Submission:

New Device

C. Measurand:

Carbon dioxide

D. Type of Test:

Quantitative

E. Applicant:

Hitachi Chemical Diagnostics, Inc.

F. Proprietary and Established Names:

S TEST Reagent Cartridge Carbon Dioxide (CO₂)

G. Regulatory Information:

Regulation Section	Classification	Product Code	Panel
21 CFR § 862.1160	Class II	KHS	Chemistry 75

H. Intended Use:

1. Intended use(s):

See Indications for Use below

2. Indication(s) for use:

The S TEST Reagent Cartridge Carbon Dioxide (CO₂) is intended for the quantitative determination of carbon dioxide concentration in serum or lithium heparin plasma using the HITACHI Clinical Analyzer E40. Carbon dioxide measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with changes in body acid-base balance. The S TEST Reagent Cartridge Carbon Dioxide (CO₂) 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:
For use with the Hitachi Clinical Analyzer E40

I. Device Description:

The S TEST Reagent Cartridge Carbon Dioxide (CO₂) has the following composition:

Reagent (1) and (2)

- PEP: Phosphoenolpyruvate, 12.5 mmol/L
- PEPC: Phosphoenolpyruvate carboxylase (Microbial), > 400 U/L
- Malate dehydrogenase (mammalian), > 4100 U/L
- NADH analog: Nicotinamide adenine dinucleotide analog, 0.6 mmol/L
- Buffer (pH 7.5 at 25°C)
- Activators, Stabilizers, Surfactant and Preservative

J. Substantial Equivalence Information:

1. Predicate device name(s):
Sekisui Diagnostics, PEI, Inc., Carbon Dioxide L3K Assay
2. Predicate 510(k) number(s):
k042362
3. Comparison with predicate:

Characteristic	Candidate device (Hitachi S TEST CO₂)	Predicate device (Sekisui CO₂)
Intended Use	Quantitative determination of carbon dioxide in human serum	Same
Instrument Platform	Hitachi Clinical Analyzer	Olympus/Beckman AU400
Testing Environment	Physician office or clinical lab	Clinical lab
Test Principle	Carbon dioxide (in the form of bicarbonate HCO ₃ ⁻) reacts with phosphoenolpyruvate in the presence of phosphoenolpyruvate carboxylase (and magnesium) to yield oxaloacetic acid (OAA). In the presence of malate dehydrogenase, reduced cofactor is oxidized by OAA. The decrease in the concentration of reduced cofactor is	Same

	monitored, and is proportional to the carbon dioxide concentration in the sample.	
Specimen Type	Human serum or plasma (Lithium Heparin)	Same
Reportable Range	5.0 to 40.0 mmol/L	2.9 to 50.0 mmol/L
Detection Wavelength	405/508 nm	405/415 nm
Detection Limit (LoQ)	1.3 mmol/L	2.9 mmol/L

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

CLSI Document EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline

CLSI Document EP6-A, Evaluation of Linearity of Quantitative Measurement Procedures, Approved Guideline

CLSI Document EP7-A2, Interference Testing in Clinical Chemistry, Approved Guideline

CLSI Document EP17-A, Protocols for the Determination of Limits of Detection and Limits of Quantitation, Approved Guideline

L. Test Principle:

The carbon dioxide (in the form of bicarbonate HCO_3^-) in the sample reacts with phosphoenolpyruvate (PEP) in the presence of phosphoenolpyruvate carboxylase (PEPC) and magnesium to yield oxaloacetic acid (OAA) and phosphate. In the second reaction, and in the presence of malate dehydrogenase (MDH), the reduced cofactor is oxidized by OAA. The reduced cofactor absorbs strongly at 405 nm, whereas its oxidized form does not. The difference in absorbance between the final reading and the blank, monitored bichromatically at 405 nm/508 nm, is directly proportional to the total carbon dioxide concentration in the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Following CLSI EP5-A2, samples (low, middle, and high levels of carbon dioxide) were tested in duplicate, twice a day, for 20 days, for a total of 80 results per level internally. The samples were commercial serum controls, including one diluted sample, to evaluate the low end measuring range. The results were tabulated and the data were analyzed for means, standard deviations (SDs), and percent coefficients of variation (%CVs). The precision results are summarized below.

Low Sample

Carbon Dioxide	Within-Run	Total
Mean (mmol/L)	10.11	10.11
SD (mmol/L)	0.13	0.45
%CV	1.3%	4.4%

Medium Sample

Carbon Dioxide	Within-Run	Total
Mean (mmol/L)	19.41	19.41
SD (mmol/L)	0.25	0.72
%CV	1.3%	3.7%

High Sample

Carbon Dioxide	Within-Run	Total
Mean (mmol/L)	33.06	33.06
SD (mmol/L)	0.40	1.22
%CV	1.2%	3.7%

POC Precision:

Studies for precision were performed at three external POL-type sites to evaluate the Hitachi E40 Clinical Analyzer with S TEST Reagent Cartridge Carbon Dioxide in one of its targeted intended use environments, the physician's office laboratory.

For the external site precision study, each site received three blinded serum samples (the Precision Panel, labeled A, B, and C) that were chosen to represent low, middle, and high concentrations of carbon dioxide. Each sample was assayed six times per day for five days, reporting 30 results per level. Precision results were as follows:

Carbon Dioxide (mmol/L)
n = 30 replicates per sample per site

Site	Sample	Mean	Within-run Precision		Total Precision	
			SD (mmol/L)	%CV	SD (mmol/L)	%CV
1	A	8.75	0.16	1.8	0.36	4.1
2	A	7.29	0.33	4.6	0.44	6.0
3	A	8.06	0.23	2.9	0.25	3.1
1	B	15.27	0.35	2.3	0.74	4.8
2	B	15.01	0.23	1.5	0.67	4.4
3	B	16.25	0.27	1.7	0.30	1.9
1	C	29.46	0.51	1.7	0.94	3.2
2	C	29.47	0.81	2.7	1.08	3.7
3	C	31.01	0.58	1.9	1.16	3.7

b. *Linearity/assay reportable range:*

A linearity study was performed according to the EP6-A guideline. Twelve (12) serial dilutions, plus the zero standard (total = 13 samples), were prepared and tested. The intermixture samples were prepared from a starting concentration of 55 mmol/L. All samples were tested in duplicate by the Hitachi Clinical Analyzer E40. The mean Hitachi results (y-axis) were plotted against the assigned values (x-axis) and a linear regression analysis was performed across the claimed measuring range.

The linear regression correlation between the expected values and the measured values for the CO₂ is summarized below:

Slope	y-intercept	r ²
0.918	0.091	0.9988

In addition, percent recovery for samples in the reportable range is shown in the table below.

Sample	Assigned	Mean	% Recovery
1	0.0	0.1	0.1 mmol/L
2	1.4	1.8	0.4 mmol/L
3	2.8	3.3	0.5 mmol/L
4	5.5	6.0	109.1%
5	11.0	11.5	104.1%
6	16.5	16.5	99.7%
7	22.0	21.9	99.5%
8	27.5	26.1	94.7%
9	33.0	32.3	97.9%
10	38.5	36.2	94.0%
11	44.0	39.9	90.7%

The sponsor's claimed range is 5 to 40 mmol/L. The percent recovery in the clinically relevant sample range of 22.0-33.0 mmol/L is within ± 5%.

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

Traceability

The CO₂ assay is traceable to American Chemical Society (ACS) reagent grade sodium carbonate alkalimetric standard.

There is no calibration needed by the user. Each lot of S TEST Reagent Cartridge Carbon Dioxide (CO₂) is calibrated by the manufacturer prior to shipment using material referenced to a standard which is traceable to American Chemical Society (ACS) reagent grade sodium carbonate alkalimetric standard. The 2D code printed on each cartridge provides the analyzer with lot-specific calibration data.

Controls

The sponsor recommends two commercially available controls for use with this assay in the labeling.

d. *Detection limit:*

Following CLSI EP17-A2 guideline, the analytical sensitivity was defined as the limit of detection (LoD), and the LoD was calculated from the limit of blank (LoB). For the LoB, the blank sample for the carbon dioxide reagent system was assayed 20 times per day for three days for a total of 60 replicate results. The LoB was estimated as the mean of the 57th and 58th highest values for the true blanks. For the LoD, five low samples were assayed four times per day for three days, for a total of 60 replicate results. The LoD was calculated as the LoB + 1.645 x SD of the low sample. The table below showed the results for the LoB, and the LoD of the carbon dioxide reagent system.

	Carbon Dioxide
LoB (mmol/L)	0.70
LoD (mmol/L)	0.89

The sponsor’s claimed measuring range of the CO₂ assay is 5 to 40 mmol/L.

e. *Analytical specificity:*

Two levels of serum based samples (low and high, approximately 17 mmol/L and 30 mmol/L, respectively) 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 by the Hitachi Clinical Analyzer E40 following CLSI EP-7A2 guideline. In each case, the spiked sample result mean was compared to its neat control mean result, and recoveries were calculated. Significant interference was defined as a change of $\geq 10\%$ in recovery. Results are summarized as follows:

Lipemia: no significant interference up to 1,000 mg/dL Intralipid

Ascorbic acid: no significant interference up to 50 mg/dL

Hemoglobin: no significant interference up to 1,000 mg/dL

Unconjugated bilirubin: no interference up to 19.1 mg/dL

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 96 serum samples including 3 spiked and 3 diluted samples spanning the measuring range (5.0 to 40.0 mmol/L) were assayed in singleton and in a blinded fashion by both the Hitachi E40 system and the Sekisui reagents on the predicate system. The comparative data were analyzed by Deming regression and are shown below.

n	r	Slope (95% CI)	y-intercept (95% CI)
96	0.981	1.03 (0.97 to 1.08)	0.98 (-0.17 to 2.12)

POC Method Comparison:

Three POL sites and a central laboratory received 47 blinded serum samples including four diluted samples and three spiked samples ranging from 5.1 to 36.8 mmol/L. Samples were assayed on the Hitachi E40 Clinical Analyzer at the POL sites using S TEST Reagent Cartridge Carbon Dioxide (y) and the predicate device as the reference method (x). Deming regression yielded the following results:

Site #	n	Range (mmol/L)	Regression Equation	“r”	CI* Slope	CI Intercept
1	47	6.6 to 36.8	$y = 0.91x + 1.49$	0.984	0.87 to 0.95	0.67 to 2.32
2	45*	5.5 to 34.4	$y = 0.92x + 0.56$	0.970	0.80 to 1.04	-2.31 to 3.43
3	47	5.1 to 35.5	$y = 0.92x + 0.79$	0.982	0.87 to 0.97	-0.43 to 2.01

* 2 samples at Site 2 quantitated below to dynamic range (<5 mmol/L) and were excluded from data analysis.

For all three POL sites, a negative bias was observed for samples above the clinically relevant range (>30 mmol/L) at POL sites. Difference between the candidate device and the predicate device is very small within the clinically relevant range.

b. Matrix comparison:

A study was performed to validate the use of lithium heparin plasma as an alternative to serum for the Hitachi Clinical Analyzer with S TEST Reagent Cartridge Carbon Dioxide. Fifty (50) matched serum/plasma samples including 2 spiked and 4 diluted samples that spanned the measuring range were assayed in singleton and the results were compared using linear regression (plasma = y-axis).

	Lithium Heparinized Plasma
Slope (95% CIs)	1.00 (0.94 to 1.05)
y-intercept (95% CIs)	-0.34 (-1.97 to 1.30)
r	0.980

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:
Reference range: 22-29 mmol/L.
Tietz, Fundamentals of Clinical Chemistry, 4th Edition, WB Saunders Company, (1996).

Each laboratory should determine the expected values for its particular population.

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.