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

A. 510(k) Number:

k180835

B. Purpose for Submission:

Modification of a previously cleared Acetaminophen assay (k081938), including a change in reagent formulation.

C. Measurand:

Acetaminophen

D. Type of Test:

Quantitative colorimetric assay

E. Applicant:

Sekisui Diagnostics PEI Inc.

F. Proprietary and Established Names:

SEKURE Acetaminophen L3K Assay

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LDP	II	21 CFR 862.3030, Acetaminophen test system	91- Toxicology

H. Intended Use:

1. Intended use(s):

   See Indications for use below.

2. Indication(s) for use:

   For the in vitro quantitative measurement of acetaminophen in serum, lithium heparin plasma and sodium heparin plasma. Measurement of acetaminophen is used in the diagnosis and treatment of acetaminophen overdose toxicity.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Roche Hitachi 717 chemistry analyzer

I. Device Description:

The SEKURE Acetaminophen L3K assay consists of two reagents, an enzyme reagent and a color reagent, and a calibrator.

- Acetaminophen Enzyme Reagent (R1): A solution containing buffer (pH 8.6 at 25°C), 0.3 mmol/L $MnCl_2 \bullet 4H_2O$, ≥ 0.9 KU/L Acyl Amidohydrolase (microbial), 50 mg/L sodium azide.
- Acetaminophen Color Reagent (R2): A solution containing 0.1 mol/L sodium carbonate buffer (pH 11.5 at 25°C), 31 mmol/L 2,5-dimethylphenol, stabilizer, preservative.
- Acetaminophen Calibrator: 1 x 5 mL of a solution containing buffer (pH 5.2 at 25°C), 151 µg/mL (1000 µmol/L) acetaminophen, preservatives.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) numbers:

   Acetaminophen L3K Assay, k081938

2. Comparison with predicate:

Similarities		
Feature	**Predicate Device (k081938): Acetaminophen L3K Assay**	**Candidate Device: SEKURE Acetaminophen L3K Assay Assay**
Intended Use	Intended for the in vitro quantitative measurement of acetaminophen in serum and plasma. Measurement of acetaminophen is used in the diagnosis and treatment of acetaminophen overdose toxicity.	Same

Similarities		
Feature	**Predicate Device (k081938): Acetaminophen L3K Assay**	**Candidate Device: SEKURE Acetaminophen L3K Assay Assay**
Test Principle	Enzymatic and Spectrophotometric	Same
Platform	Hitachi 717	Same

Differences		
Feature	**Predicate Device (k081938): Acetaminophen L3K Assay**	**Candidate Device: SEKURE Acetaminophen L3K Assay Assay**
Sample Types	Serum and lithium heparin plasma	Serum, lithium heparin plasma and sodium heparin plasma
Measuring interval	4 to 2500 µmol/L (0.6 to 377.5 µg/mL)	115 to 2500 µmol/L (17.4 to 377.5 µg/mL)

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

- CLSI EP05-A3- Evaluation of Precision Performance of Quantitative Measurement Methods
- CLSI EP06-A- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
- CLSI EP07-A2- Interference Testing in Clinical Chemistry
- CLSI EP17-A2- Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
- CLSI EP09-A3- Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition
- CLSI EP25-A- Evaluation of Stability of In Vitro Diagnostic Reagents

L. Test Principle:

The enzyme, acyl amidohydrolase, cleaves the amide bond of the acetaminophen molecule, leaving p-aminophenol and acetate. The p-aminophenol is coupled with 2,5-dimethylphenol in the presence of manganese ions to form a colored compound, 4-(4-iminophenol)-2,5-dimethylcyclohexadiene-1-one. The increased absorbance at 605 nm due to the formation of 4-(4-iminophenol)-2,5-dimethylcyclohexadiene-1-one is directly proportional to the concentration of acetaminophen in the sample.

M. Performance Characteristics (if/when applicable):

All performance studies were conducted with the Roche Hitachi 717 clinical chemistry analyzer.

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed using two unaltered patient serum samples, two spiked patient serum samples and three levels of controls, assayed in duplicate, twice a day, for 20 days, with daily calibration. Each sample was evaluated using six lots of Acetaminophen L3K Assay.

Repeatability:

Lot	Sample	N	Mean Acetaminophen (µmol/L)	Repeatability		Within Laboratory	
				SD (µmol/L)	%CV	SD (µmol/L)	%CV
1	Control 1	80	68	1.8	2.7	2.5	3.8
	Control 2	80	238	4.4	1.9	6.1	2.6
	Control 3	80	764	13.4	1.8	14.3	1.9
2	Unaltered P1	80	170.3	2.1	1.2	2.4	1.4
	Unaltered P2	80	195.5	2.4	1.2	2.6	1.4
	Spiked 1	80	1295.9	11.5	0.9	19.7	1.5
	Spiked 2	80	2278.6	17.3	0.8	51.0	2.2
3	Control 1	80	68	1.1	1.7	2.4	3.5
	Control 2	80	238	3.9	1.7	5.8	2.4
	Control 3	80	764	14.2	1.9	14.2	1.9
4	Unaltered P1	80	169.5	1.5	0.9	2.1	1.2
	Unaltered P2	80	195.4	2.6	1.3	2.6	1.3
	Spiked 1	80	1294.3	12.8	1.0	21.4	1.7
	Spiked 2	80	2281.5	15.9	0.7	52.3	2.3
5	Control 1	80	68	1.4	2.0	2.4	3.5
	Control 2	80	238	4.0	1.6	5.8	2.4
	Control 3	80	764	13.6	1.7	14.2	1.8
6	Unaltered P1	80	175.0	1.6	0.9	2.0	1.2
	Unaltered P2	80	202.0	2.7	1.3	3.0	1.5
	Spiked 1	80	1339.3	13.8	1.0	22.5	1.7
	Spiked 2	80	2357.9	17.5	0.7	50.0	2.1

b. Linearity/assay reportable range:

Linearity was conducted in a study using three lots of SEKURE Acetaminophen L3K reagent and two lots of calibrator. Nine samples from 100 to 2700 µmol/L acetaminophen were prepared. A high concentration stock was prepared from a stock acetaminophen standard of USP acetaminophen in pooled acetaminophen-free human serum. The high concentration stock was then diluted with an acetaminophen free sample to generate seven linearity samples. Expected concentrations were calculated based on the known value of the USP standard material values and the dilution factors. Samples were analyzed in quadruplicate. The mean and deviation of the

measured mean from theoretical values were calculated.

Expected Acetaminophen (µmol/L)	Lot 1		Lot 2		Lot 3	
	Observed (µmol/L)	Deviation (%)	Observed (µmol/L)	Deviation (%)	Observed (µmol/L)	Deviation (%)
100	98.8	-1.2%	99.3	-0.7%	101.3	1.3%
115	116.3	1.1%	115.5	0.4%	118.5	3.0%
125	125.3	0.2%	124.5	-0.4%	128.8	3.0%
250	251.0	0.4%	251.5	0.6%	260.3	4.1%
500	489.5	-2.1%	493.0	-1.4%	509.8	2.0%
1000	1015.8	1.6%	1022.8	2.3%	1048.8	4.9%
2000	2018	0.9%	2020.0	1.0%	2079.5	4.0%
2500	2515	0.6%	2501.3	0.0%	2587.3	3.5%
2700	2690.3	-0.4%	2686.5	-0.5%	2784.5	3.1%

The linearity data met acceptance criteria and support a measuring range from 115 to 2500 µmol/L (17.4 to 377.5 µg/mL).

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

The SEKURE Acetaminophen Calibrator is traceable to USP grade reference acetaminophen material. Stability protocol and results are reviewed and found acceptable. Stability testing supports a shelf life of 12 months.

d. *Detection limit:*

Limit of Blank (LoB): LoB was evaluated according to CLSI EP17-A2 using three lots of SEKURE Acetaminophen L3K reagent on the Hitachi 717. Five blank samples were assayed over three operating days for a total of 60 replicates per sample. For each reagent lot, the LoB results were ranked from lowest to highest concentration, and the LoB was estimated to be the result equivalent to the concentration at the 95th percentile. The LoB was determined to be 1.0 µmol/L.

Limit of Detection (LoD): Five low level samples were evaluated using three lots of reagents over three operating days for a total of 60 replicates per sample. The LoD was calculated for each reagent lot by $LoD = LoB + C_pSD_L$. This study yielded a LoD of 2.4 µmol/L.

Limit of quantitation (LoQ): A set of six low-level samples was measured using three lots of reagents over five runs across three operating days for a total of 40 replicates per sample. The LoQ was calculated based on % total error is <25%. The LoQ was determined to be 8 µmol/L.

e. *Analytical specificity:*

i. Interference from endogenous substances/cross reactants

Interference studies were performed in accordance to CLSI EP07-A2. Four acetaminophen serum pools prepared from USP acetaminophen dissolved in patient serum pool (33 µmol/L, 100µmol/L, 199 µmol/L and 900 µmol/L) were evaluated. Testing was conducted using three lots of Acetaminophen reagent and two lots of calibrator on the Hitachi 717. The potential interferents were added individually to aliquots of the serum specimen pools. Control samples contained corresponding concentrations of acetaminophen without potential interferents. Significant interference was evaluated using a significance criterion of greater than ±10% or 8 µmol/L from control. The highest concentrations of each substance that did not show significant interference with each acetaminophen concentration tested are shown in the table below.

Substance concentration Tested	Highest Tested Concentration with No Significant Interference	Acetaminophen Concentration
Hemoglobin (0 - 1000 mg/dL)	200 mg/dL (31 µmol/L)	30 µmol/L (4.5 µg/mL)
	200 mg/dL (31 µmol/L)	93 µmol/L (14.0 µg/mL)
	400 mg/dL (62 µmol/L)	188 µmol/L (28.4 µg/mL)
	1000 mg/dL (155 µmol/L)	861 µmol/L (130 µg/mL)
Conjugated Bilirubin (0 - 40 mg/dL)	2 mg/dL (23.7 µmol/L)	31 µmol/L (4.7 µg/mL)
	2 mg/dL (23.7 µmol/L)	98 µmol/L (14.8 µg/mL)
	32 mg/dL (364 µmol/L)	115 µmol/L (17.4 µg/mL)
	40 mg/dL (475 µmol/L)	200 µmol/L (30.2 µg/mL)
	40 mg/dL (475 µmol/L)	957 µmol/L (144 µg/mL)
Unconjugated Bilirubin (0 - 40 mg/dL)	2 mg/dL (34.2 µmol/L)	33 µmol/L (5.0 µg/mL)
	4 mg/dL (68 µmol/L)	98 µmol/L (14.8 µg/mL)
	32 mg/dL (547 µmol/L)	115 µmol/L (17.4 µg/mL)
	24 mg/dL (410 µmol/L)	200 µmol/L (30.2 µg/mL)
	40 mg/dL (475 µmol/L)	958 µmol/L (144 µg/mL)
Ascorbic Acid (0 - 3000 µg/dL)	3000 µg/dL (170 µmol/L)	30 µmol/L (4.5 µg/mL)
	3000 µg/dL (170 µmol/L)	107 µmol/L (16.2 µg/mL)
	3000 µg/dL (170 µmol/L)	215 µmol/L (32.5 µg/mL)
	3000 µg/dL (170 µmol/L)	946 µmol/L (142.8 µg/mL)
N-Acetylcysteine (0 - 2500 mg/L)	1500 mg/L	30 µmol/L (4.5 µg/mL)
	1500 mg/L	110 µmol/L (16.6 µg/mL)
	1500 mg/L	215 µmol/L (32.5 µg/mL)
	1500 mg/L	922 µmol/L (139.2 µg/mL)
Intralipid (0 – 1000 mg/dL)	600 mg/dL [1800 mg/dL (20 mmol/L) Simulated Triglycerides]	32 µmol/L (4.8 µg/mL)
	1000 mg/dL [3000 mg/dL (34 mmol/L) Simulated	100 µmol/L (15.1 µg/mL)

Substance concentration Tested	Highest Tested Concentration with No Significant Interference	Acetaminophen Concentration
	Triglycerides]	
	1000 mg/dL [3000 mg/dL (34 mmol/L) Simulated Triglycerides]	203 µmol/L (30.7 µg/mL)
	1000 mg/dL [3000 mg/dL (34 mmol/L) Simulated Triglycerides]	898 µmol/L (135.6 µg/mL)

Interference from common drugs was also examined. A total of 15 commonly used drugs were examined for potential interferences. Testing was performed with serum sample pools at four levels of acetaminophen (33, 100, 199, and 900 µmol/L). Recoveries of acetaminophen were within the sponsor's acceptance criteria of greater than ±10% or 8 µmol/L from control. The highest concentrations of common drugs shown not to interfere with the assay are provided in the table below.

Substance Tested	Concentration with No Significant Interference
Theophylline	222 µmol/L
Phenylbutazone	2.89 mmol/L
Ibuprofen	2425 µmol/L
Imipramine	2.5 µmol/L
Acetylsalicylic Acid	6.51 mmol/L
Levodopa	25.3 µmol/L
Ampicillin	152 µmol/L
Doxycycline	67.5 µmol/L
Amitriptyline	3.61 µmol/L
Metronidazole	701 µmol/L
Cefoxitin	1546 µmol/L
Cyclosporine	10.0 µmol/L
Methyldopa	71 µmol/L
Rifampicin	78.1 µmol/L
Salicylate	4.34 mmol/L

f. Assay cut-off:

Not applicable.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      A method comparison test was conducted on the Roche Hitachi 717 analyzer using three lots of SEKURE Acetaminophen L3K reagent, as compared to one lot of the predicate Acetaminophen L3K reagent. 105 samples were tested in singlicate. The samples tested ranged from 116 to 2447 µmol/L (17.5 -369.4 µg/mL). Deming regression analysis resulted in a slope of 0.974, a y-intercept of 4.7 µmol/L, and a correlation coefficient of 0.9999. Passing-Bablok regression analysis resulted in a slope of 0.975, a y-intercept of 2.3 µmol/L, and a correlation coefficient of 0.9999.

   b. *Matrix comparison:*

      Matrix comparison studies were performed for Serum (with gel in plastic, SST), EDTA, Lithium heparin (with gel in plastic, PST), Lithium heparin (without gel in plastic), Lithium heparin (Barricor tube), Sodium heparin (without gel in plastic) vs serum (without gel in plastic). A set of fifty matched sample sets were tested and percent recovery was determined. All percent recoveries were within ± 10%.

	Sample Range	Slope		Correlation Coefficient
		Result Deming	Passing-Bablok	Result
SST	115 – 2322 µmol/L	1.012	1.007	1.000
EDTA		0.303	0.303	0.988
Lithium Heparin		1.015	1.012	1.000
PST		1.009	1.000	1.000
Sodium Heparin		1.012	1.006	1.000
Barricor		1.005	1.000	1.000

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 were cited from the following literatures: Tietz Textbook of Clinical Chemistry, Second Edition, pp 1168, 2212, W.B. Saunders Company, Philadelphia (1994)

Therapeutic concentration: 10-30 µg/mL (66-199 µmol/L)
Toxic concentration: > 200 µg/mL (1324 µmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish its own expected range.

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.