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

A. 510(k) Number:
k141928

B. Purpose for Submission:
New Device

C. Measurand:
Acetaminophen

D. Type of Test:
Quantitative enzyme immunoassay

E. Applicant:
Roche Diagnostics

F. Proprietary and Established Names:
cobas C Acetaminophen Gen.2 assay
ACET2 Calibrator

G. Regulatory Information:

1. Regulation section:
   21 CFR Sec. 862.3030-Acetaminophen test system
   21 CFR Sec. 862.3200-Clinical toxicology calibrator

2. Classification:
   Class II

3. Product code:
   LDP-Colorimetry, Acetaminophen
   DKB-Calibrators, Drug Mixture

4. Panel:
   Toxicology (91)

H. Intended Use:

1. Intended use(s):
   See Indications for use below.

2. Indication(s) for use:

The cobas c Acetaminophen Gen.2 assay is an in vitro diagnostic test for the quantitative determination of acetaminophen of acetaminophen overdose in serum and plasma on Roche/Hitachi cobas c systems.

The ACET2 calibrator is for use in the calibration of the Acetaminophen Gen.2 Roche assay.

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   Roche Hitachi cobas c 501 clinical chemistry analyzer

I. Device Description:

The cobas c Acetaminophen Gen.2 assay consists of reagents packaged in a cassette labeled with their instrument positioning, R1 (Reagent 1) and R2 (Reagent 2).

- R1 contains anti-acetaminophen antibody (sheep polyclonal), Glucose-6-phosphate, Nicotinamide adenine dinucleotide (NAD), bovine serum albumin, preservatives (Proclin), and stabilizers (pepstatin and aprotinin).

- R2 contains acetaminophen labeled with bacterial Glucose-6-phosphate dehydrogenase, Tris buffer, preservatives (Sodium Azide and 2-methyl-2H-isothiazol-3-one (MIT)) bovine serum albumin, and stabilizers (pepstatin and aprotinin).

The ACET2 calibrator contains a known quantity of acetaminophen (~200 µg/mL), sodium phosphate, sodium EDTA, sodium chloride (NaCl), and preservatives (Proclin and MIT). The cobas c 501 analyzer dilutes the ACET2 calibrator on-board the analyzer with NaCl diluent, in order to create five concentration levels (level 2, 3, 4, 5, and 6) containing 10, 30.2, 75.0, 100, and 200 µg/mL of acetaminophen, respectively. Level 1 contains no acetaminophen.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Emit® tox™ Acetaminophen Assay
   Emit® tox™ Acetaminophen Calibrators

2. Predicate 510(k) number(s):
   K002974

3. Comparison with predicate:

Assay Comparison - Similarities and Differences		
Feature	**Predicate Device: Emit® tox™ Acetaminophen Assay**	**Candidate Device: Acetaminophen Gen.2**
Intended Use	The Emit® tox™ Acetaminophen Assay is a homogeneous enzyme immunoassay intended for in vitro diagnostic use in the quantitative analysis of acetaminophen in human serum or plasma.	Same
Test Principle	Homogeneous enzyme immunoassay	Same
Measuring Range	Up to 200 µg/mL (1324 µmol/L)	5−200 µg/mL (33.1−1324 µmol/L)
Traceability	This method has been standardized against USP reference standards.	Same
Sample Types	Serum and EDTA, heparin, citrate and oxalate/fluoride plasma	Serum and K_2− or K_3−EDTA, or lithium heparinized plasma

Calibrator Comparison - Similarities and Differences		
Feature	**Predicate Device: Emit® tox™ Acetaminophen Calibrators**	**Candidate Device: ACET2 Calibrator**
Intended Use	The Emit® tox™ Acetaminophen Calibrators are intended for use with the Emit® tox™ Acetaminophen Assay.	ACET2 calibrator is for use in the calibration of the Acetaminophen Gen.2 Roche assay.
Format	Liquid ready-to-use	Same
Analyte	Acetaminophen	Same
Storage Conditions	2-8°C	Same

Storage and Stability	When stored refrigerated at 2–8°C, the Emit® tox™ Acetaminophen Calibrators are stable until the expiration date printed on the dropper vial label.	Same
Quantity	Emit® tox™ Acetaminophen Calibrators are a six-level set that contain the following acetaminophen concentrations: 0, 10, 25, 50, 100, 200 µg/mL.	The ACET2 calibrator has a nominal value of 200 µg/mL of acetaminophen. The analyzer dilutes the ACET2 calibrator on-board with NaCl diluent, in order to create five concentration levels. This results in a six- level calibrator set: 0.0, 10.0, 30.2, 75.0, 100, 200 µg/mL.

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

- Standard Title CLSI EP05-A2- Evaluation of Precision Performance of Quantitative Measurement Methods
- Standard Title CLSI EP06-A2- Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
- Standard Title CLSI EP17-A2- Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures
- Standard Title CLSI EP09-A2- Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition

L. Test Principle:

The assay is based on a homogeneous enzyme immunoassay technique used for the quantitative analysis of acetaminophen in human serum or plasma. The assay is based on competition between drug in the sample and drug labeled with the enzyme glucose-6-phoshate dehydrogenase (G6PDH) for antibody binding sites. Enzyme activity decreases upon binding to the antibody, so the drug concentration in the sample can be measured in terms of enzyme activity. Active enzyme converts oxidized nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that is measured spectrophotmetrically (at 415 nm and 340 nm).

M. Performance Characteristics (if/when applicable):

All performance studies were conducted with the Roche Hitachi cobas c 501 clinical chemistry analyzer.

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

Precision of the Acetaminophen Gen.2 assay was evaluated with a 21-day precision study according to CLSI EP05-A2, using control material as well as pooled human sera samples (from patients taking acetaminophen). The acetaminophen concentrations of all tested samples are noted in the table below. All samples were tested using 3 reagent kit lots, with 2 replicates per run and 2 runs per day. Data from all lots tested were similar. Data from a representative lot is presented below.

Test Specimen	Mean Acetaminophen Concentration (µg/mL)	Within-Run (Repeatability)		Between-Run		Total Precision (within lab precision)	
		SD (µg/mL)	% CV	SD (µg/mL)	% CV	SD (µg/mL)	% CV
Human Sample 1	7.6	0.224	3.0	0.107	1.4	0.258	3.4
Human Sample 2	73	1.66	2.3	0.314	0.4	1.95	2.7
Human Sample 3	130	2.90	2.2	0	0	3.32	2.5
Human Sample 4	169	3.69	2.2	0.377	0.2	4.66	2.8
Human Sample 5	184	4.25	2.3	0	0	5.31	2.9
Control 1	15.3	0.385	2.5	0.193	1.3	0.455	3.0
Control 2	35.3	0.637	1.8	0.470	1.3	0.791	2.2
Control 3	107	2.18	2.0	0.641	0.6	2.71	2.5

b. Linearity/assay reportable range:

Linearity was assessed in a study using serum and Li-Heparin plasma samples containing acetaminophen concentrations across the measuring range of the assay (5-200 µg/mL acetaminophen). Samples were prepared by 13 dilutions of a high concentration sample with an acetaminophen free sample. Expected concentrations were determined based on the known value of the commercially available standard material values and the dilution factors. Samples were analyzed in triplicate. Percent recoveries relative to the expected concentrations are shown below for serum and Li-Heparin plasma. Results support linearity across the measuring range.

Serum

Sample	Expected (µg/mL)	C501 mean (µg/mL) (µg/mL)	Absolute deviation (µg/mL)	% recovery of mean values
1	0	0.00	0.0	-
2	3	2.40	-0.6	-
3	6	5.40	-0.6	90
4	24	23.1	-	96
5	48	45.7	-	95
6	72	68.4	-	95
7	96	92.1	-	96
8	12	113	-	94
9	14	139	-	97
10	168	160	-	95
11	192	188	-	98
12	216	206	-	95
13	240	232	-	97

Li-Heparin plasma

Sample	Expected (µg/mL)	c 501 mean (µg/mL)	Absolute deviation (µg/mL)	% recovery of mean values
1	0	0	0	-
2	3	2.4	-0.6	-
3	6	5.1	-0.9	85
4	24	21.7	-	90
5	48	44.3	-	92
6	72	65.4	-	91
7	96	89.0	-	93
8	120	110	-	92
9	144	132	-	92
10	168	155	-	92
11	192	180	-	94
12	216	194	-	90
13	240	228	-	95

Dilution Accuracy:

The sponsor provided data supporting dilution accuracy (re-run function) by comparing manually diluted serum and Li-heparin plasma samples to expected values (5-fold dilution). Each sample was diluted with NaCl. Data is provided in the table below.

Sample type	Replicate	Expected acetaminophen concentration (µg/mL)	Auto rerun acetaminophen concentration (µg/mL)	Mean Expected value	Mean autorerun value	% Recovery
Serum	1	234	232.0	224.7	230.7	102.7
	2	224	226.7			
	3	216	233.4			
Serum	1	510	566.8	535.7	561.3	104.8
	2	551	560.1			
	3	546	557.0			
Serum	1	839	871.3	839.0	887.1	105.7
	2	817	907.4			
	3	861	882.5			
Plasma	1	201	209.7	206.7	212.0	102.6
	2	213	213.7			
	3	206	212.6			
Plasma	1	614	629.2	611.7	625.7	102.3
	2	615	614.2			
	3	606	633.7			
Plasma	1	773	852.9	794.3	854.7	107.6
	2	785	873.2			
	3	825	838.1			

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

The cobas c Acetaminophen Gen.2 assay is calibrated using a calibrator that was previously cleared under k002974. The Roche ACET2 calibrator is prepared gravimetrically from commercially available purified materials. See k002974 for traceability and stability information.

d. Detection limit:

LoB, LoD, and LoQ studies were performed based upon CLSI EP17-A2.

LoB Protocol
One analyte-free sample was measured in replicates of 5 for 2 runs on 2 analyzers for 3 days for each of 3 reagent lots (for total n=60 per lot). The LoB was determined to be 1.5 µg/mL .

LoD Protocol
Five low-analyte samples spiked with acetaminophen were measured with 1 determination per run (2 runs) on two analyzers with 3 reagent lots for six runs per day across three days. This study yielded a LoD of 3 µg/mL.

LoQ Protocol

A set of six low-level samples was measured in two aliquots using 3 lots over at least 6 days (n=12 for each low-level sample). The total number of measurements made for each lot was 72.

Bias was determined by the difference between average and target values. The target values were determined by a chromatography system. Standard deviations were determined over all replicates for each lot. The following results were observed.

Lot	Mean value (ug/mL) (C501)	SD (ug/mL)	Absolute bias compared to target value (ug/mL)
1	4.73	0.123	0.367
2	4.63	0.129	0.475
3	4.88	0.221	0.217

The LoQ was determined to be 5 µg/mL.

e. Analytical specificity:

i. Interference from endogenous substances/cross reactants

Cross reactivity with compounds of similar structure were evaluated by using serum sample pools, with two acetaminophen target concentrations (~5.0 and ~30.0 µg/mL). Test samples included the acetaminophen plus potential cross-reactant (at the concentrations shown below). Control samples contained corresponding concentrations of acetaminophen without potential interferents. The table below provides the % cross reactivity for each of the compounds tested.

Compound	Compound Concentration (µg/mL)	Acetaminophen (µg/mL)	% cross reactivity
Acetaminophen cysteine	100	6.1	0.5
Acetaminophen glucuronide	1000	5.2	n.d.*
Acetaminophen mercapturate	300	5.4	0.2
Acetaminophen sulfate	200	6.1	n.d.*
Phenacitin	500	6.7	0.5
Acetaminophen cysteine	100	29.2	-0.3
Acetaminophen glucuronide	1000	25.4	-0.1

Acetaminophen mercapturate	300	25.9	0.2
Acetaminophen sulfate	200	27.8	0.1
Phenacitin	500	29.3	1.3

The n.d.* stands for 'not detectable'.

The assay was also evaluated with hemoglobin (hemolysis), lipids (lipemia), and bilirubin (Icterus) for potential interferences with the measurement of acetaminophen using serum sample pools. Evaluation of interference included normal levels of bilirubin and lipids (triglycerides). Recoveries of acetaminophen were within the sponsor's acceptance criteria of ± 10%. No interference was noted when tested up to the concentrations shown in the table below. The H and I index values in the table are equivalent to ~1mg/dL.

	Conc. Of Acetaminophen (µg/mL)	No interference up to
Lipemia Level 1	6.3	1100 mg/dL
Lipemia Level 2	29.7	997 mg/dL
Lipemia Level 3	52.7	810 mg/dL
Lipemia Level 4	104	698 mg/dL
Lipemia Level 5	149	682 mg/dL
Lipemia Level 6	178	678 mg/dL
Hemolysis Level 1	5.8	926 H index
Hemolysis Level 2	28.2	936 H index
Hemolysis Level 3	46.1	1009 H index
Hemolysis Level 4	91.8	1008 H index
Hemolysis Level 5	135	1016 H index
Hemolysis Level 6	174	817 H index
Unconjugated bilirubin Level 1	6.2	47 I index
Unconjugated bilirubin Level 2	30.2	45 I index
Unconjugated bilirubin Level 3	51.1	63 I index
Unconjugated bilirubin Level 4	99.1	63 I index
Unconjugated bilirubin Level 5	150	63 I index
Unconjugated bilirubin Level 6	178	63 I index
Conjugated bilirubin Level 1	6.5	45 I index
Conjugated bilirubin	30.3	46 I index

Level 2		
Conjugated bilirubin Level 3	50.7	44 I index
Conjugated bilirubin Level 4	99.9	47 I index
Conjugated bilirubin Level 5	148	50 I index
Conjugated bilirubin Level 6	181	42 I index

ii. Interference from common drugs

Interference from common drugs was also examined. A total of 24 commonly used drugs were examined for potential interferences. Testing was performed with serum sample pools at two levels of acetaminophen (~7 and ~30 µg/mL). Recoveries of acetaminophen were within the sponsor's acceptance criteria of ± 10%. The highest concentrations of common drugs shown not to interfere with the assay are provided in the table below.

Drug	Highest Concentration Shown Not to Interfere with ACET2
Acetylcysteine	1663 µg/mL
Acetylsalycilic acid	1000 mg/L
Ampicillin-sodium	1000 mg/L
Ascorbic acid	300 mg/L
Cefoxitin	2500 mg/L
Cyclosporine	5 mg/L
Doxycycline	50 mg/L
Phenylbutazone	400 mg/L
Rifampicin	64 mg/L
Theophylline	100 mg/L
Amitriptylline	277 µg/mL
Caffeine	1000 µg/mL
Codeine	1.6 µg/mL
Diazepam	5.1 µg/mL
Heparin	5000 U/mL
Ibuprofen	500 mg/L
Levodopa	20 mg/L
Methyldopa	20 mg/L
Metronidazole	200 mg/L
Methionine	1000 µg/mL
Phenylephrine	20 µg/mL
Propoxyphene	20 µg/mL
Salicylate	1000 µg/mL

Secobarbital	22 µg/mL
Imipramine	280 µg/mL
Cysteine	1300 µg/mL

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison test was performed on the Roche/Hitachi cobas c 501 analyzer by testing 117 human serum samples in singlicate on the Acetaminophen Gen.2 assay and the predicate device. The samples ranged in concentration from 5.2 to 198 µg/mL. An additional set of eleven native serum samples from individual patients taking acetaminophen were spiked with a stock solution of 4 mg/mL acetaminophen in NaCl so concentrations were above the measuring range of the assay. These samples were spiked with a minimal volume of the stock solution, so that dilution effects were minimized. Deming regression analysis resulted in a slope of 1.02, a y-intercept of -0.699, and a correlation coefficient of 0.997. Ninety-five percent confidence intervals for the y-intercept and slope were -0.981 to -0.417 and 1.005 to 1.030, respectively.

b. Matrix comparison:

Matrix comparison studies were performed for Li-Heparin plasma vs serum, K_2-EDTA plasma vs serum, and K_3-EDTA plasma vs. serum. A total of 50 tubes were collected per anticoagulant and percent recovery was determined. All percent recoveries were within ± 10%.

anticoagulants	Sample concentration range tested (µg/mL)	Claimed Measuring Range (µg/mL)
Li-Heparin	2.6 - 187	5 – 200
K_2-EDTA (full)	2.6 - 187	
K_3-EDTA (full)	2.6 - 187	

In addition, the following linear regression statistics were obtained (Passing/Bablok):

Matrix	slope	y-intercept	r value
Lithium-heparin vs serum	0.989	0.0891	0.998
K_2-EDTA vs serum	1.000	0.000	0.998
K_3-EDTA vs serum	0.992	-0.163	0.998

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:

   Normal therapeutic doses of acetaminophen result in serum concentrations of 10-30 µg/mL (66-199 µmol/L) in healthy adults. (From Jacobs DS, DeMott WR, Grad HI, et al. Laboratory Test Handbook 5th ed. Stow, Ohio: Lexi Comp Inc 2001;768).

   Toxic manifestations have been observed at serum concentrations > 100 µg/mL , however the toxic range is generally reported at > 200 µg/mL . Toxic concentrations can be more effectively related to post dose interval; > 200, > 100, and > 50 µg/mL serum concentrations at 4, 8, and 12 hours post dose, respectively. (Rumack BH. Acetaminophen overdose. Arch Intern Med 1981 ;141 :380)

   Each laboratory should investigate the transferability of the expected values of its own patient population and if necessary determine its own reference ranges.

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.