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

A. 510(k) Number:

k100200

B. Purpose for Submission:

New device

C. Measurand:

Acetaminophen

D. Type of Test:

Quantitative colorimetric assay

E. Applicant:

Siemens Healthcare Diagnostics Inc.

F. Proprietary and Established Names:

ADVIA® Chemistry Acetaminophen Reagent

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3030 – Acetaminophen test system

2. Classification:

   Class II

3. Product code:

   LDP

4. Panel:

   91 Toxicology

H. Intended Use:

1. Intended use(s):

   See indications for use below

2. Indication(s) for use:
   For *in vitro* diagnostic use in the quantitative determination of acetaminophen in human serum and plasma (lithium heparin) on ADVIA Chemistry systems. Such measurements are used in the detection of acetaminophen overdose.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   ADVIA 1650 analyzer

I. Device Description:

The Acetaminophen reagents are ready-to-use liquid reagents (150 tests/wedge, four Reagent 1 and four Reagent 2 wedges / kit) containing:

Reagent 1 (R1) – buffer, Manganese Chloride, Acyl Amidohydroxylase, 0.09% w/v sodium azide

Reagent 2 (R2) – buffer, 8-Hydroxyquinoline-5-sulfonic acid

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Genzyme Diagnostics (formerly DCL) Acetaminophen Assay

2. Predicate 510(k) number(s):
   k042330

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Indications for Use	For in vitro diagnostic use in the quantitative determination of	same

Similarities		
Item	Device	Predicate
	acetaminophen in human serum and plasma (lithium heparin). Measurements are used in the detection of acetaminophen overdose.	
Test principle	conversion of acetaminophen to produce p-aminophenol by the action of acylamidohydrolase. The p-aminophenol is converted to a colored product by reaction with 8-hydroxyquinoline sulfonic acid. The increased absorbance is directly proportional to the concentration of acetaminophen in the sample.	same
Format	Liquid	same

Differences		
Item	Device	Predicate
Reportable range	0.2 to 20.0 mg/dL	0.3 to 38.0 mg/dL

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

CLSI - EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The enzyme, acyl amidohydrolase, cleaves the amide bond of the acetaminophen molecule, leaving p-aminophenol and acetate. The p-aminophenol reacts with 8-hydroxoquinoline-5-sulfonic acid in the presence of manganese ions to form a colored compound 5-(4-iminophenol)-8-quinoline. The increased absorbance at 596/751 nm is directly proportional to the concentration of acetaminophen in the sample.

M. Performance Characteristics (if/when applicable):

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

a. *Precision/Reproducibility:*

Precision estimates were computed according to CLSI document EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods. 10 days of precision studies were performed. Within run and total imprecision were evaluated by testing 3 individual, normal serum pools spiked with acetaminophen. In addition, 2 levels of a serum-based control material and a single-level calibrator (acetaminophen in human serum albumin) were tested. The acetaminophen levels were chosen to challenge the upper and lower limits of the expected range of the assay, the medically significant cut-off level as well as various levels throughout the assay range. Each sample was assayed 2 times per run and 2 runs per day for 10 days, totaling 40 replicates per system. The experiment was performed using one reagent lot on one ADVIA 1650 system. One operator performed the study.

			Within-run		Total	
Sample #	n	Level	SD	CV (%)	SD	CV (%)
1	40	1.28	0.01	1.1	0.00	1.1
2	40	2.79	0.02	0.9	0.04	1.6
3	40	8.99	0.04	0.4	0.08	1.0
4	40	9.57	0.05	0.5	0.11	1.3
5	40	15.62	0.06	0.4	0.14	1.0
6	40	18.45	0.06	0.3	0.17	1.0

An additional precision study was performed over a period of 10 days (2 runs per day – total 20 runs) using 2 reagent lots on 2 ADVIA 1650 systems and incorporating 2 calibrations on each system. Within run and total imprecision were evaluated by testing 4 individual, normal serum pools spiked with acetaminophen. In addition, 2 levels of a serum-based control material were tested. Each sample was assayed 2 times per run and 2 runs per day for 10 days. The study was run using two reagent lots on two ADVIA 1650 systems (both lots run on both instruments).

			Within-run		Total	
Sample	n	Level	SD	CV (%)	SD	CV (%)
Serum pool 1	160	0.21	0.02	10.8	0.02	12.0
Serum pool 2	160	0.75	0.01	1.9	0.02	2.7
Control 1	160	1.35	0.01	0.9	0.02	1.8
Serum pool 3	160	18.18	0.10	0.5	0.20	1.1
Control 2	160	9.43	0.06	0.6	0.11	1.1
Serum pool 4	160	3.22	0.02	0.6	0.04	1.2

b. *Linearity/assay reportable range:*

The measuring range of the assay is 0.2 – 20 mg/dL. Nine (9) diluted samples were prepared from high and low serum samples by dilution. Fresh normal human serum was spiked with approximately 23 mg/dL of Acetaminophen, in order to provide a sample just above the desired upper range of the assay. The same serum, un-spiked, which contained no acetaminophen, was used as a low sample. The deviation was calculated as the difference of the expected value and the mean observed result of 2 replicates. Percent deviation for all acetaminophen-containing tested pools was less than 1.7% throughout the range of the assay.

Linear regression: y = 1.0014 x - 0.0090

b = 1.0014 (95% Confidence interval 0.9967 - 1.0060)

a = -0.0090 (95% Confidence interval -0.0643 - 0.0462)

r = 1.000

The results of the study demonstrated that the acetaminophen assay is linear up to the sponsor's stated claim of 0.2 to 20 mg/dL.

Automatic rerun conditions for this method extend the analytical range to 60 mg/dL. An acetaminophen-free serum sample was spiked to approximately 60 mg/dL (3 x times the analytical range of the ADVIA assay) with purified acetaminophen. A series of dilutions of this sample was made using an un-spiked acetaminophen-free sample. The expected concentration of the samples above the range of the assay was calculated based on the values within the assay range times dilution factor. The sample was assayed with automatic high-range rerun enabled. This data was generated using one system and one reagent lot with each level assayed in duplicate (one operator)

The highest level tested gave an ADVIA value of 61.7 mg/dL which is 101.2% of the expected value of 61.0 mg/dL.

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

ADVIA Chemistry Acetaminophen assay is calibrated using ADVIA Chemistry ToxAmmonia calibrators cleared previously under k023184. The ADVIA Chemistry Acetaminophen method is traceable to an internal standard manufactured using purified material.

d. Detection limit:

The sponsor set the Limit of Quantitation (LoQ) of the assay at 0.2 mg/dL which is consistent with the low end of the linearity and precision studies (see 1. a. above).

e. Analytical specificity:

The sponsor tested potential interferents up to the indicated levels and the results shown in the table below. The sponsor defined < 10 % interference as not significant in this evaluation.

ADVIA 1650			
Interferent	**Interferent level**	**ACET Sample Concentration**	**Interference ***
Bilirubin (conjugated)	15 mgdL (257 umol/L)	3.4 mg/dL (224 umol/L)	NSI
	20 mgdL (342 umol/L)	3.4 mg/dL (224 umol/L)	12%
	5 mg/dL (86 umol/L)	1.7 mg/dL (112 umol/L)	NSI
	10 mg/dL (171 umol/L)	1.7 mg/dL (112 umol/L)	14%
Bilirubin (unconjugated)	5 mg/dL (86 umol/L)	3.4 mg/dL (224 umol/L)	NSI
	10 mg/dL (171 umol/L)	3.4 mg/dL (224 umol/L)	11%
	5 mg/dL (86 umol/L)	1.7 mg/dL (112 umol/L)	12%
Hemolysis (hemoglobin)	250 mg/dL (2.5 g/L)	3.4 mg/dL (224 umol/L)	NSI
	500 mg/dL (5.0 g/L)	3.4 mg/dL (224 umol/L)	13%
	250 mg/dL (2.5 g/L)	1.7 mg/dL (112 umol/L)	13%
Lipemia (from Intralipid)	250 mg/dL (2.8 mmol/L)**	3.4 mg/dL (224 umol/L)	NSI
	500 mg/dL (5.7 mmol/L)**	3.4 mg/dL (224 umol/L)	-11%
	250 mg/dL (2.8 mmol/L)**	1.7 mg/dL (112 umol/L)	NSI
	500 mg/dL (5.7 mmol/L)**	1.7 mg/dL (112 umol/L)	-18%

* NSI = No Significant Interference. A percentage effect ≥10% is considered a significant interference.
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The table is included in the labeling. The sponsor states in the labeling that visibly hemolyzed or lipemic samples should not be tested and that significant interference may occur at very low concentrations of acetaminophen with hemolyzed, lipemic, or icteric samples.

Specific therapy for acetaminophen overdose is to administer N-acetylcysteine (NAC, Mucomyst). The potential for NAC interference in the acetaminophen assay was examined. N-acetylcysteine (NAC) at the level up to 1000 mg/L was spiked in a serum sample pool with approximate acetaminophen concentration of 10 mg/dL. Multiple levels of NAC were tested. The tested samples were prepared by various dilutions of a pool with the highest concentration of interferent with the same pool without interferent (control). Calculations were performed by comparing observed Acetaminophen concentration at each level of interferent vs. observed Acetaminophen concentration of control (no interferent) expressed in %. No significant effect of NAC, defined by the sponsor, as < 10 % interference was seen up to 800 mg/dL. A -20% effect was seen at a level of 1000 mg/dL NAC. The following information is included in the labeling:

> *Interference from N-acetylcysteine (NAC) was evaluated on ADVIA Chemistry system. Using a significance criterion of >10% variance from control, acceptable results were obtained to a concentration of 800 mg/L N-acetylcysteine (NAC) in a 10.2 mg/dL acetaminophen sample; this in-vitro analysis was performed approximately two hours after the addition of NAC to a serum pool. Note: Significantly reduced Acetaminophen recovery has been demonstrated in situations where testing has been performed immediately after the introduction of NAC. It is recommended that laboratories review NAC treatment and monitoring protocols to determine the extent of the potential interference.*

The sponsor tested the effect of potentially interfering drugs using 2 human serum pools with acetaminophen concentrations of approximately 0.5 mg/dL and 3.0 mg/dL. A single level of interferent was tested at the concentration in the table below. The method was considered by the sponsor to have no significant interference (NSI) if a bias between control and observed acetaminophen concentration is < 10%. The table below is included in the sponsor's labeling.

Substance Tested	Concentration Tested	Effect at 0.5 mg/dL Acetaminophen*	Effect at 3 mg/dL Acetaminophen*
Acetylsalicylic Acid	6.5 mmol/L	NSI	NSI
Amitriptyline	3.6 µmol/L	NSI	NSI
Ampicillin	152 µmol/L	NSI	NSI
Ascorbic Acid	342 µmol/L	NSI	NSI
Cefoxitin	1546 µmol/L	NSI	NSI
Doxycycline	67.5 µmol/L	NSI	NSI
Ibuprofen	2425 µmol/L	NSI	NSI
Imipramine	2.5 µmol/L	NSI	NSI
Levodopa	25.3 µmol/L	NSI	NSI
Methyl-L-Dopa	71 µmol/L	NSI	NSI
Metronidazole	701 µmol/L	NSI	NSI
Phenylbutazone	2.9 mmol/L	NSI	NSI
Rifampicin	78.1 µmol/L	+38.9%	NSI
Salicylate	4.3 mmol/L	NSI	NSI
Theophylline	222 µmol/L	NSI	NSI

*NSI = No Significant Interference.
A percentage effect ≥10% is considered a significant interference.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A total of 95 serum samples were analyzed in singlicate on the ADVIA Chemistry 1650 system using ADVIA Chemistry Acetaminophen method vs. the Genzyme Acetaminophen method to demonstrate the equivalence of the two methods. The study was conducted using one lot of Genzyme Acetaminophen reagent and one lot of ADVIA Chemistry Acetaminophen reagent. One trained operator performed the study. Serum samples ranged from 0.4 to 19.5 mg/dL. These values span the proposed measuring range of the assay (0.2 - 20 mg/dL). The linear regression equation from the study yielded the following results:

Linear regression: y = 1.021x (95% confidence intervals = 1.0161 – 1.0265) + 0.18,(95% confidence intervals = 0.13 – 0.22); r = 1.000

b. Matrix comparison:

Serum / plasma equivalency studies were performed to characterize the correlation between serum and lithium heparin plasma samples. 24 matched serum and plasma (Li-heparin) samples were collected in-house. The paired

serum and plasma pairs (from same donor) were spiked with identical levels of purified acetaminophen to achieve concentrations of acetaminophen ranging from 0.75 to 18.01 mg/dL. The samples were analyzed with the ADVIA Chemistry Acetaminophen method on one system using one lot of reagent, in singlicate. Results were analyzed using linear regression. The linear regression equation from the study yielded the following result:

$y + 1.01x – 0.03$, $r = 1.000$

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):

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:

   Existing literature reference was used:

   1. Tietz Clinical Guide to Laboratory Tests, 4th edition, Saunders Elsevier, St. Louis, MO:2006:1238.
   2. Merck Manual, http://www.merck.com/mmpe/sec21/ch326/ch326c.html#BGBHJFCE

   Therapeutic Range 1–2 mg/dL (66–132 μmol/L)

   Toxic Concentration 4 hours post-ingestion >15 mg/dL (993 μmol/L)

   12 hours post-ingestion >4 mg/dL (265 μmol/L)

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.