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

A. 510(k) Number:

k083386

B. Purpose for Submission:

New Device

C. Measurand:

Calcium

D. Type of Test:

Quantitative, colorimetric assay

E. Applicant:

Siemens Healthcare Diagnostics

F. Proprietary and Established Names:

ADVIA Chemistry Calcium_2 Method

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
CJY	Class II	21 CFR§ 862.1145	Clinical Chemistry (75)

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 calcium in human serum, plasma, and urine on the ADVIA Chemistry systems. Such measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal failure, and tetany.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   ADVIA 1650 Chemistry system

I. Device Description:

The device is sold as two options: option 1 - 7x40mL wedges (38mL fill each) in a kit, option 2 - 8x70mL wedges (68mL fill each) in a kit. Reagents are provided ready to use. The reagent component concentrations are as follows: Sodium acetate, pH 5.9, 54.2 mmol/L, Arsenazo III 188 µmol/L, and non-reactive stabilizers.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Calcium method for the Bayer ADVIA 1650 system (currently Siemens)

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

   k991576

3. Comparison with predicate:

Similarities		
Characteristics	**Candidate Device:** Siemens ADVIA Calcium_2	**Predicate Device:** Bayer ADVIA 1650 Calcium
Intended Use	For *in vitro* diagnostic use in the quantitative determination of calcium in human serum, plasma, and urine on the ADVIA Chemistry systems. Such measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal failure, and tetany.	same
Sample Type	Serum, plasma (Li-heparin) and urine	Serum, plasma (Li-heparin) and urine
Instrument	ADVIA® Chemistry 1650 system	ADVIA® Chemistry systems
Calibrators	Siemens Chemistry Calibrator (k030169)	Same (k030169)
Controls used	BioRad, or other commercial controls	BioRad, or other commercial controls
Method	colorimetry	colorimetry

Differences		
Characteristics	Siemens ADVIA Calcium_2	Bayer ADVIA 1650 Calcium
Assay Protocol	Calcium ions form a colored complex with Arsenazo III, which is measured at 658/694 nm. The amount of calcium present in the sample is directly proportional to the intensity of the colored complex formed. ***Reaction Equation*** *Ca^{2+} + Arsenazo III ---> Ca-Arsenazo III Complex (purple)*	Calcium ions form a violet complex with o-cresolphthalein complexone in an alkaline medium. The reaction is measured at 545/658 nm. ***Reaction Equation*** *CPC + 2 Ca^{2+} ---> CPC $(Ca^{2+})_2$ Complex*
Reagents	One liquid reagent	Two liquid reagents
Measuring Range *	<u>Serum/Plasma:</u> 1.0 – 16.0 mg/dL (0.25 – 4.0 mmol/L) <u>Urine:</u> 1.0 – 32.0 mg/dL (0.25 – 8.0 mmol/L)	<u>Serum/ Plasma:</u> 1.0 – 15.0 mg/dL (0.25 – 3.75 mmol/L) <u>Urine:</u> 1.0 – 30.0 mg/dL (0.25 – 7.50 mmol/L)
Interfering Substances **	Bilirubin–NSI to 50 mg/dL Hemoglobin–NSI up to1000 mg/dL Lipemia (Intralipid)–NSI to 1000 mg/dL	Bilirubin–NSI to 30 mg/dL Hemoglobin–NSI up to 525 mg/dL Lipemia (Intralipid)–NSI to 650 mg/dL
Precision *	2.1% at 5.8 mg/dL (serum) 1.3% at 9.8 mg/dL (serum) 0.8% at 13.8 mg/dL (serum) 3.0% at 6.0 mg/dL Ca (urine) 2.1% at 23.6 mg/dL Ca (urine)	2.7% at 5.9 mg/dL (serum) 2.9% at 10.8 mg/dL (serum) 3.5% at 12.0 mg/dL (serum) 2.4% at 6.2 mg/dL (urine) 2.5% at 21.2 mg/dL Ca (urine)
Accuracy / Correlation *	<u>Serum :</u> y = 0.98 x + 0.44; $S_{y,x}$= 0.21; r=0.993 (vs. ADVIA 1650 Ca)	<u>Serum :</u> y = 0.99 x + 0.13; $S_{y,x}$ = 0.22; r = 0.971 (vs. Technicon DAX)

	Urine : y = 0.97 x + 0.30; $S_{y,x}$=0.34 ; r= 0.998 (vs. ADVIA 1650 Ca)	Urine : y = 1.07 x + 0.03; $S_{y,x}$ = 0.56 ; r = 0.988 (vs. Beckman CX3)
Traceability	Traceable to Inductively Coupled Plasma Atomic Emission, which uses reference materials from the NIST ***	Traceable to the NIST*** atomic absorption reference method

* - data for both devices shown from ADVIA 1650/1800 performance
** - NSI – No Significant Interference
*** - National Institute of Standards and Technology

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

- CLSI EP17-A – Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline
- CLSI EP05-2A – Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline- 2nd Edition
- Format for Traditional and Abbreviated 510(k)s – Guidance for Industry and FDA Staff
- In Vitro Diagnostic Devices: Guidance for the Preparation of 510(k) Submissions

L. Test Principle:

The Calcium_2 (CA_2) method is based on the work of Michaylova and Illkova, who found that Arsenazo III could form a stable complex with calcium with high selectivity at low pH. The ADVIA 1650 system automatically predilutes the sample with saline by 5-fold. The sample predilution allows for very small sample volumes to be used for multiple tests run in random access on the analyzer. From the diluted sample, 4 uL is added to 100 uL of the reagent, mixed, and incubated for 5 minutes at 37C. Calcium ions form a colored complex with Arsenazo III, which is measured at 658 nm. Absorbances of test samples are compared with that of the Calibrator to convert signal into calcium concentrations reported to the customer. The amount of calcium present in the sample is directly proportional to the intensity of the colored complex formed.

Reaction Equation: Ca^{2+} + Arsenazo III ----- > Ca-Arsenazo III Complex (purple)

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

   Precision was evaluated using CLSI document EP05-A2 as a guideline. Samples from two serum controls, two urine controls, one spiked serum pool, and one spiked, urine pool were tested. Each sample was assayed 2 times per

run, 2 runs per day for 10 days, totaling 40 replicates using the ADVIA 1650 system. The experiment was run using one reagent lot on two systems for serum and one reagent lot on one system for urine. Medical decision levels, as well as normal range and abnormal range of the assay were challenged in this experiment.

The precision data are summarized as follows:

			Within Run		Total	
Product	**Mean (mg/dL)**	**N**	**SD**	**%CV**	**SD**	**%CV**
serum (control)	5.82	40	0.08	1.4	0.12	2.1
serum (control)	9.81	40	0.12	1.3	0.13	1.3
serum (serum pool, spiked)	13.83	40	0.07	0.5	0.11	0.8
urine (control)	5.95	40	0.07	1.2	0.18	3.0
urine (control)	11.80	40	0.11	0.9	0.18	1.6
urine (urine pool, spiked)	23.61	40	0.11	0.5	0.50	2.1

b. *Linearity/assay reportable range:*
Linearity was evaluated by comparing observed values versus expected values for 9 equally-spaced diluted samples prepared from high and low pools (separately for serum and urine). Each sample was measured in replicates of 3 using the ADVIA 1650 analyzer. The percent recovery for serum samples was within 100 – 105.5% and for urine samples it was within 100 – 106.1%.

In addition, the expected values (X) were plotted against the observed values (Y) and a line fit was plotted. The linear regression for serum samples is Y= 1.0026X + 0.1679, r= 0.9998. For urine samples, the linear regression is Y = 0.9971X = 0.3775, r = 0.9998.

The data provided supported the sponsor's claim that this assay has a reportable range of 1 – 16 mg/dL for serum samples and 1 – 32 mg/dL for urine samples.

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

Traceability:
The ADVIA CA_2 method is traceable to an internal Siemens reference method (Inductively Coupled Plasma Atomic Emission), which uses reference

materials from the National Institute of Standards and Technology (NIST), via patient sample correlation.

Stability:
The reagent has an on-board stability of 30 days on the ADVIA 1200, ADVIA 1650/1800, and ADVIA 2400 systems. Unopened reagents are stable until the expiration date when stored at 15 – 25°C. Reagents should not be frozen.

d. Detection limit:

The limit of the blank (LoB) and limit of detection (LoD) were determined in accordance with the guidelines of CLSI document EP17-A using the ADVIA 1650 analyzer. Deionized water, low serum control material and low urine sample material were used to determine the LoB and LoD. Functional sensitivity of the assay was not calculated. Testing was conducted over a period of 10 days, with 2 runs per day using one system and one reagent lot. A total of 40 replicates of the blank sample, and 40 replicates of the low samples were run. The results are as follows:

LoB (serum) = 0.12 mg/dL
LoB (urine) = 0.09 mg/dL
LoD (serum) = 0.31 mg/dL
LoD (urine) = 0.25 mg/dL

The assay has a reportable range of 1 – 16 mg/dL for serum samples and 1 – 32 mg/dL for urine samples.

e. Analytical specificity:

i. Interference from endogenous substances:

Potential interfering substances [unconjugated and conjugated bilirubin, triglycerides (using Intralipid and avian TRIG), and hemoglobin] were spiked into two pools of human serum samples. The calcium concentrations of the two pools were 6 mg/dL and 12 mg/dL. Two chelated Gadolinium contrast agents were also tested for interference, gadodiamide (Omniscan) and gadoversetamide (Optimark). Unspiked aliquots served as the control, and the analysis was performed using an ADVIA 1650 analyzer. The sponsor claims no significant interference if the % recovery is < 10% between the tested and the control samples. Percent recoveries ranged from 100 to 109.8%. No significant interference was observed for the following levels of interferents:

Interferent	Concentration
Hemoglobin	1000 mg/dL
Bilirubin (Unconjugated)	50 mg/dL
Bilirubin (Conjugated)	50 mg/dL
Triglycerides (avian TRIG)	1000 mg/dL
Triglycerides (Intralipid)	1000 mg/dL
Gadolinium (Omniscan)	2.0 mmol/L
Gadolinium (Optimark)	2.0 mmol/L

ii. Cross-reactivity:
None referenced

f. Assay cut-off:
Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
A method comparison study was performed using 172 serum samples ranging from 1.10 mg/dL to 15.55 mg/dL and 50 urine samples ranging from 1.15 mg/dL to 25.76 mg/dL. Two serum samples were excluded for not being within the measuring range of the assay. The studies were performed on the ADVIA 1650 analyzer using the CA_2 assay (new device) and the CA assay (predicate).
The regression correlation is summarized as follows:

ADVIA 1650 CA_2 (Candidate Device = y) *vs.* ADVIA 1650 CA (Predicate Device = x)

Sample	Regression Equation	Syx, mg/dL	r	n	Range, m/dL
Serum	Y = 0.96x + 0.70	0.27	0.996	170	1.10 – 15.55
Urine	Y = 0.96x + 0.29	0.34	0.998	50	1.15 – 25.76

b. Matrix comparison:
A matrix comparison study was performed using 25 paired serum and Li-heparinized plasma samples containing calcium across the assay range. Samples above the normal reference range were spiked. Testing was performed using one ADVIA 1650 analyzer on one reagent lot over 2 days. One sample was beyond the range of the assay and was excluded from the calculations.
The linear regression correlations are summarized as follows (plasma = y, serum = x):

Regression Equation	S_{yx}, mg/dL	r	n	(x)Range, mg/dL
Y = 0.996x – 0.15	0.24	0.996	24	2.00-15.28

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:

   The serum/plasma reference range was determined by using 148 serum samples from apparently healthy adult volunteers (77 females and 71 males). The samples were assayed in duplicate on the ADVIA 1650 system using one reagent lot with non-parametric analysis to calculate the values at the 2.5 and 97.5 percentile. These results show that 95 percent of specimens fell within the calcium concentrations of 8.7 – 10.4 mg/dL (2.18 – 2.60 mmol/L), with samples ranging from 8.5 – 10.8 mg/dL (2.13 – 2.7 mmol/L). Since the equivalency between serum and plasma samples with the CA_2 method was shown in a matrix comparison study (section M.2.b), this reference range applies to both serum and plasma samples.
   For urine ranges, a literature reference was used (Tietz NW. *Clinical Guide to Laboratory Tests.* Third Edition. Philadelphia, PA: WB Saunders Company; 1995:102-105). Expected results are as follows:

Matrix	Range (mg)	Range (mmol)
Serum/Plasma	8.7 – 10.4 (mg/dL)	2.18 – 2.60 (mmol/L)
Urine	100 – 300 (mg/day)	2.5 – 7.5 (mmol/day)

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.