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

A. 510(k) Number:
k113521

B. Purpose for Submission:
New device

C. Measurand:
Calcium

D. Type of Test:
Quantitative photometric method

E. Applicant:
Roche Diagnostics

F. Proprietary and Established Names:
Calcium Gen. 2

G. Regulatory Information:

Measurand	Regulation Section	Classification	Product Code	Panel
Calcium	21CFR862.1145	Class II	CHW-Photometric	(75) Clinical Chemistry

H. Intended Use:

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

2. Indication(s) for use:
   The Calcium Gen.2 assay is an in vitro diagnostics reagent system intended for the quantitative determination of calcium in human serum, plasma, and urine on Roche/Hitachi cobas c systems. Calcium measurements are used in the diagnosis and treatment of parathyroid disease, a variety of bone diseases, chronic renal disease, and tetany.

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

3. Special instrument requirements:

   All performance studies were conducted on the cobas c501 analyzer.

I. Device Description:

The Calcium Gen. 2 test system consists of the following reagents:

R1 CAPSO:[a] 557 mmol/L; NM-BAPTA: 2 mmol/L; pH 10.0; non-reactive surfactant; preservative.
R2 EDTA: 7.5 mmol/L, pH 7.3; non-reactive surfactant; preservative

a) 3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid

J. Substantial Equivalence Information:

1. Predicate Device Name(s):
   Roche Calcium

2. Predicate 510(k) number(s):
   k921661

3. Comparison with predicate:

Similarities and Differences		
Item	Candidate Device Calcium Assay	Predicate Device (k921661) Calcium Reagent
Intended Use	Same	In vitro test for the quantitative determination of calcium in human serum, plasma and urine on Roche automated clinical chemistry analyzers.
Specimen Type	Serum, Li-Heparin Plasma and Urine	Serum, Heparin Plasma, and Urine
Reagent Type	Liquid ready-for-use	Same
Assay Principle	photometric test	Same
Instrument	cobas c 501 analyzer	Roche Hitachi analyzers
Calibrators	Same	Calibrator f.a.s.
Calibration Mode	Same	Two point linear

Controls	Precinorm U Plus Precipath U Plus Precinorm U Precipath U PreciControl ClinChem Multi1 PreciControl ClinChem Multi2	· Precinorm U Plus · Precipath U Plus · Precinorm U · Precipath U
Reagent Active Ingredients	5-nitro-5'-methyl-BAPTA	o-Cresolphthalein complexone, 8-hydroxyquinoline, HCl acid
Reagent Stability	**Unopened:** 2-8°C until expiration date **On-board in use:** 42 days	**Unopened** 15-25°C until expiration date **On-board in use** R1: 42 days R2: 90 days
Measuring Range	**Serum/Plasma:** 0.8 – 20.1 mg/dL (0.2 – 5.0 mmol/L) **Urine:** 0.8 – 30.1 mg/dL (0.2 to 0.75 mmol/L)	**Serum/Plasma:** 0.2 – 20 mg/dL **Urine:** 0.48 – 48 mg/dL

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

- CLSI Guideline, EP5-A2 *Evaluation of Precision Performance of Clinical Chemistry Devices – Second Edition*
- CLSI Guideline, EP6-A2 *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*
- CLSI Guideline, EP17-A2 *Protocols for Determination of Limits of Detection and Limits of Quantitation*

L. Test Principle:

The Calcium Gen. 2 test system employs a photometric test method where calcium ions react with a calcium specific polyamino carboxylic acid under alkaline conditions to form a complex. This complex reacts in the second step with EDTA. The calcium concentration is directly proportional to the change in absorbance which is measured photometrically.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   All the performance studies were conducted using the cobas c501 analyzer.

a. *Precision/Reproducibility:*

Precision was evaluated according to the CLSI Document EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*. This study assessed repeatability (within-run precision), and intermediate precision (total precision based on between-day, between-run, and within-day). Imprecision of the assay was established by assessing human serum pool and urine pool with one reagent lot, two runs per day for 21 days on one cobas c501 analyzer with one lot of previously cleared calibrators. Results are summarized below:

Serum:

	Total Precision				Within Run			
	N	Mean- mg/dL (mmol)	SD	% CV	N	Mean- mg/dL (mmol/L)	SD	%CV
Human Serum 01	84	2.4 (0.60)	0.02	2.5	21	2.4 (0.59)	0.01	1.7
Human Serum 02	84	10.2 (2.55)	0.02	0.9	21	10.0 (2.51)	0.02	0.6
Human Serum 03	84	17.8 (4.46)	0.04	0.9	21	17.7 (4.44)	0.02	0.4
Control 1	84	9.0 (2.25)	0.02	0.8	21	9.0 (2.24)	0.02	0.7
Control 2	84	14.0 (3.51)	0.03	0.8	21	14.0 (3.51)	0.03	0.9

	Total Precision				Within Run			
	N	Mean- mg/dL (mmol/L)	SD	% CV	N	Mean- mg/dL (mmol/L)	SD	%CV
Human Urine 01	84	2.32 (0.58)	0.02	3.1	21	2.28 (0.57)	0.02	2.6
Human Urine 02	84	15.7 (3.92)	0.05	1.2	21	15.5 (3.88)	0.03	0.8
Human Urine 03	84	17.8 (4.46)	0.04	0.9	21	20.6 (5.15)	0.03	0.5
Human Urine 04	84	24.4 (6.09)	0.08	1.3	21	24.4 (6.09)	0.05	0.8
Control 1	84	9.0 (2.25)	0.02	0.8	21	7.5 (1.88)	0.20	1.0
Control 2	84	14.0 (3.51)	0.03	0.8	21	10.9 (2.72)	0.02	0.8

b. *Linearity/assay reportable range:*

The measuring range of the assay is 0.8 to 20.1 mg/dL (0.20 - 5.0 mmol/L) for serum and plasma samples and 0.8 to 30.1 mg/dL (0.20 - 7.5 mmol/L) for urine samples. High analyte level serum and urine samples were diluted with 0.9% NaCl to produce eleven concentrations across the measuring ranges (serum samples were in the range of 0.5 to 20. 6; urine samples were in the range of 0.4 to 32.2). Calcium levels were measured in triplicate. The measured vs. expected linear regression analysis for all sample types generated a linear regression as follows:
Serum: $y=1.000x-0.000$ $r^2=0.999$
Urine: $y=1.0028x-0.0152$ $r^2=0.999$

Results of the study support the sponsor's claim that the assay is linear from 0.8 to 20.1 mg/dL for serum and plasma samples and 0.8 to 30.1 mg/dL for urine.

For urine sample only: Users can select automatic re-run with dilution for samples above 30 mg/dL on the cobas c501 analyzer. A dilution factor of 5 is automatically applied. There is no automatic re-run function available for serum and plasma samples Therefore, the extended measuring range of urine samples using automated rerun with dilution was validated by performing an experiment comparing the instrument auto-rerun results with a simple manual dilution. Two cobas c 501 analyzers were used in this experiment. Three samples with calcium concentrations > 30 mg/dL were manually diluted in triplicate per analyzer. Sample medians were compared to the instrument auto-rerun results. Percent recovery was calculated. Results from this study were within 90% to 110% recovery.

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

Calibrators were cleared in k062319 .
Precinorm U Plus and Precipath U Plus were previously cleared in k042389.
PreciControl ClinChem Multi 1 and Multi 2 were previously cleared in k102016.

The sponsor performed reagent stability studies and protocol and acceptance were reviewed and found to be adequate. Accelerated stability studies were performed to simulate 24 months stability of the reagent when stored at 2°C to 8°C. Real time stability studies are still on-going.

d. *Detection limit:*
The Limit of the Blank (LoB) and Limit of Detection were established following the CLSI guideline EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*. The analyte free urine and serum samples were used for determination of LoB values. Each sample was

measured with 3 reagent lots in 5-fold determinations in 6 runs, distributed over 3 days on 2 cobas c 501 analyzers. In total, 60 determinations were obtained per reagent lot. Data analysis was based on determination of the 95th percentile of the 60 measured values. The LoB was determined to be 0.24 mg/dL (0.06 mmol/L) for urine and 0.12 mg/dL (0.03) mmol/L for serum.

5 samples with low-analyte concentration (serum samples and urine samples) were used in determination of LoD values. Specifically, the low analyte urine and serum samples were each measured with 3 reagent lots in 1-fold determinations in 6 runs, distributed over 3 days on 2 cobas c501 analyzers. The sponsor concludes that these results support a performance claim of LoB= 0.4 mg/dL (0.10 mmol/L) and LoD= 0.8 mg/dL (0.20 mmol/L) for serum and urine.

The LoQ was determined in accordance with CLSI-EP17 A requirements. For LoQ, a low level sample set was prepared by diluting 3 human serum samples with analyte free diluent (0.9% NaCl). The sample set was measured with 3 reagent lots in 1-fold determination in 6 runs, distributed over 3 days on 2 cobas c 501 analyzers. In total, 180 determinations were obtained per reagent lot. To determine the LoQ, the differences between the expected values and the measured mean value (n=12) were calculated for each member of the low level sample set to determine the total error. The LoQ is derived from a plot on the allowable error versus the expected calcium value at a total error of less than 15% for serum and less than 25% for urine. LoQ was determined to be 0.8 mg/dL for both serum and urine.

The Ca Gen.2 assay has the measuring range of 0.8 to 20.1 mg/dL for serum and 0.8 to 30.1 mg/dL for urine.

e. Analytical specificity:

The sponsor performed studies to evaluate effect of endogenous interferences for icterus (conjugated and unconjugated bilirubin), hemolysis (hemoglobin), lipemia (intralipids), and magnesium and exogenous compounds on the performance of the Calcium Gen. 2 assay, following CLSI EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline*. Testing was done in the presence of two levels of Calcium (~8 mg/dL and ~ 16.4 mg/dL) and different concentrations of the listed compounds. Percent recovery was calculated relative to control samples (serum and urine) containing Calcium without spiked compounds. The sponsor defines non-significant interference as <10% difference between the spiked and the control samples. Based on the results, the sponsor concludes the following:

Serum:

Icterus: no significant interference up to an I index of 60
Hemolysis: no significant interference up to and H index of 1000
Lipemia: no significant interference up to an L index of 1000
Magnesium: no significant interference up to 15 mmol/L

Urine:
Icterus: no significant interference up to a conjugated bilirubin concentration of 60 mg/dL
Hemolysis: no significant interference up to a hemoglobin concentration of 1000 mg/dL
Magnesium: no significant interference up to a concentration of 60 mmol/L

Exogenous substances were tested at 2 concentrations. The tables below list all exogenous substances tested at concentrations with non-significant (<10%) interference:

Serum:

Substance	**Concentration with <10% interference (mg/dL)**
Acetylcysteine	1.5
Ampicillin-Na	10
Ascorbic acid	3.0
Cyclosporine	0.05
Cefoxitin	25
Heparin	5000 U
Levodopa	0.2
Methyldopa	0.2
Metronidazole	2
Phenylbutazone	4
Doxycycline	0.5
Acetylsalicylic acid	10
Rifampicin	0.6
Acetaminophen	2
Ibuprofen	5
Theophyllin	1

Urine:

Substance	**Concentration with <10% interference (mg/dL)**
Acetaminophen	3
N-Acetyl cysteine	0.1
Salicyluric acid	60
Ascorbic acid	40
Ampicillin-Na	10
Cefoxitin	25
Cyclosporine	120
Gentamycine Sulfate	4

Ibuprofen	40
Levodopa	10
Methyldopa	20
Ofloxacine	9
Phenazopyridine	3
Tetracycline	3

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

The sponsor performed studies to compare the performance of the Calcium assay with the performance of the predicate assay (k921661). 69 native serum and native urine samples in the range of 1.12 to 18.6 mg/dL and 1.4 to 29.0 mg/dL were used in this comparison. Results of regression analysis are summarized below:

Serum:

Regression analysis	Intercept	Slope	Confidence Interval (95%)			
	a	b	a lower	a upper	b lower	b upper
Passing Bablok	-0.0268	1.0180	-0.0525	-0.0008	1.0069	1.0285

Linear regression:

Urine:

Regression analysis	Intercept	Slope	Confidence Interval (95%)			
	a	b	a lower	a upper	b lower	b upper
Passing Bablok	0.0184	1.0237	-0.0363	0.0407	1.0161	1.030

b. Matrix comparison:

The sponsor performed a matrix study to compare the performance of the assay when different sample types/tubes (serum vs. LiHeparin plasma) were tested. A total of 60 unaltered patient samples were tested in the range of 1.44 to 18.0 mg/dL. Samples were analyzed on ten cobas c 501 analyzers. Each plasma samples was compared to the respective serum samples. Results of regression analysis are summarized below:

Regression analysis	Intercept	Slope	Confidence Interval (95%)			
	a	b	a lower	a upper	b lower	b upper
Passing Bablok	-0.0027	0.9914	-0.02	0.0245	0.9806	1.0

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 ranges:
Serum/plasma:

Adults (18-60 years): 2.15-2.50 mmol/L (8.6-10.0 mg/dL)
Adults (60-90 years): 2.20-2.55 mmol/L (8.8-10.2 mg/dL)
Adults (> 90 years): 2.05-2.40 mmol/L (8.2-9.6 mg/dL)

Wu AHB, ed. Tietz Clinical Guide to Laboratory Tests, 4th ed. St. Louis (MO): Saunders Elsevier 2006:202–207

N. Proposed Labeling:

The labeling is sufficient and does satisfy 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.