510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K131605

B. Purpose for Submission:

New Device

C. Measurand:

IgG antibodies to Cytomegalovirus (CMV)

D. Type of Test:

Electrochemiluminescence immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys CMV IgG Assay
Elecsys PreciControl CMV IgG

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.3175- Cytomegalovirus serological reagents

2. Classification:

   Class II

3. Product code:

   LFZ

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Elecsys CMV IgG assay is an in vitro qualitative test for the detection of IgG antibodies to CMV in human serum, lithium-heparin plasma, K_2-EDTA plasma, and K_3-EDTA plasma. The test is intended as an aid in the determination of the serological status to CMV in individuals in which a CMV IgG test was ordered, including pregnant women.

   Performance characteristics have not been evaluated in immunocompromised or immunosuppressed individuals. This test is not intended for use in neonatal screening or for use at point of care facilities. This test is not intended for use in screening blood and plasma donors.

   The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

   Elecsys PreciControl CMV IgG is used for quality control of the Elecsys CMV IgG immunoassay on the Elecsys and cobas e immunoassay analyzers.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   Not Applicable

4. Special instrument requirements:

   The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

I. Device Description:

The Elecsys CMV IgG assay is a two-step sandwich immunoassay which uses streptavidin microparticles, biotinylated recombinant CMV-specific antigen labeled with a ruthenium complex, and electrochemiluminescence detection. The results are determined using a calibration curve generated by two-point calibration and a master curve provided via the reagent bar code. Results greater than or equal to 1.0 COI are considered reactive for CMV IgG antibody. The test system contains the human serum-based calibrators intended for use with the system.

The Elecsys PreciControl CMV IgG contains liquid control serum based on human serum. The controls are used for monitoring the accuracy of the Elecsys CMV IgG assay.

The reagents and calibrators are packaged together in the Elecsys CMV IgG assay kit, while

the associated Elecsys PreciControl CMV IgG is packaged separately.

The following Reagents are provided in the Elecsys CMV IgG assay kit:

1. The reagent rackpack consists of reagents: M, R1, and R2 and is labeled as CMVIGG:

   - M: Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservatives: MIT (0.1%) and Oxypyrion (0.1%).

   - R1: CMV Ag~biotin (gray cap), 1 bottle, 9 mL: Biotinylated CMV-specific antigen (recombinant, *E. coli*) > 400 µg/L, MES buffer 50 mmol/L, pH 6.5; preservatives: MIT (1%) and Oxypyrion (1%).

   - R2: CMV Ag~Ru(bpy) (black cap), 1 bottle, 9 mL: CMV specific antigen (recombinant, *E. coli*) labeled with ruthenium complex > 400 µg/L; MES buffer 50 mmol/L, pH 6.5; preservatives: MIT (1%) and Oxypyrion (1%).

2. CMVIGG Cal1: Negative calibrator 1 (white cap), 2 bottles of 1.0 mL each: Human serum, non-reactive for anti-CMV IgG; buffer; preservatives: MIT (0.1%) and Oxypyrion (0.1%).

3. CMVIGG Cal2: Positive calibrator 2 (black cap), 2 bottles of 1.0 mL each: Human serum, reactive for anti-CMV IgG, approx. 40 COI; buffer; preservatives: MIT (0.1%) and Oxypyrion (0.1%).

The following are the Materials that are required but not provided:

1. PreciControl CMV IgG, 5 x 1 mL each of PreciControl CMV IgG 0, 1 and 2.
2. 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles.
3. General laboratory equipment.
4. Elecsys 2010, MODULAR ANALYTICS E170 or cobas e analyzer.
5. Accessories for Elecsys 2010 and cobas e 411 analyzers.
6. Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers.

J. Substantial Equivalence Information:

1. Predicate device name:
   Is-CMV IgG Test System

2. Predicate 510(k) number:
   K981163

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use/ Indications for Use	Intended as an aid in the determination of the serological status to CMV.	same
Analyte	IgG antibodies to CMV	Same

Differences		
Item	Device	Predicate
Assay Technology	Electrochemiluminescent Immunoassay	Enzyme-linked immuno-adsorbent assay
Sample Types	human serum, lithium-heparin plasma, K_2-EDTA plasma, and K_3-EDTA plasma	serum

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

- Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition. CLSI document EP5-A2, (7-110) Volume 24, No. 25, August 2004.
- User Verification of Performance for Precision and Trueness – Second Edition. CLSI document EP15-A2, September 2008.
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. CLSI document EP 17-A, Volume 24, No. 34, October 2004

L. Test Principle:

The test utilizes a sandwich principle. The total duration of the assay is 18 minutes and involves the following steps:

- 1st incubation: 20 µL of sample, biotinylated recombinant CMV-specific antigens, and CMV-specific recombinant antigens labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is generated by two-point calibration

and a master curve provided via the reagent barcode.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Intra-laboratory Precision:

Precision was determined using Elecsys reagents, samples and controls in a protocol based on CLSI document EP5-A2. Human serum samples and assay controls were measured in single determinations in four separate aliquots, one run per day for 21 days. The measurements were performed on the Elecsys 2010 and MODULAR ANALYTICS E170 analyzers, each at one site, with one reagent lot, performing calibration according to the instructions for use. The following results were obtained:

Elecsys CMV IgG Intra-laboratory Precision

Elecsys 2010 and cobas e 411 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean (COI) [a]	SD [b] (COI)	CV %	SD (COI)	CV %
PC CMV IgG 0 [c]	0.001	0.004	-	0.005	-
PC CMV IgG 1	1.40	-	0.9	-	3.2
PC CMV IgG 1	1.36	-	1.6	-	3.3
PC CMV IgG 2	24.6	-	0.7	-	3.3
PC CMV IgG 2	24.0	-	1.4	-	3.1
HS 1[d]	0.857	-	1.5	-	3.2
HS 2	0.971	-	1.9	-	3.4
HS 01	1.17	-	1.2	-	2.6
HS 02	1.17	-	1.5	-	3.3
HS 3	1.25	-	1.4	-	3.3
HS 03	10.4	-	1.4	-	3.0
HS 04	54.8	-	1.5	-	3.9
HS 05	212	-	1.4	-	3.6
HS 06	437	-	1.5	-	3.3

[a] COI: Cut off index
[b] SD: Standard deviation
[c] PC: PreciControl
[d] HS: human serum

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean (COI)	SD (COI)	CV %	SD (COI)	CV %
PC CMV IgG 0	0.001	0.003	-	0.004	-
PC CMV IgG 1	1.38	-	1.0	-	3.2

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean (COI)	SD (COI)	CV %	SD (COI)	CV %
PC CMV IgG 1	1.43	-	2.0	-	4.2
PC CMV IgG 2	24.1	-	1.1	-	3.7
PC CMV IgG 2	25.2	-	1.6	-	4.2
HS 1	0.875	-	1.7	-	4.0
HS 2	1.05	-	1.5	-	4.3
HS 01	1.11	-	1.4	-	3.2
HS 02	1.14	-	1.7	-	4.0
HS 3	1.28	-	1.7	-	4.0
HS 03	10.3	-	1.5	-	3.4
HS 04	53.1	-	1.3	-	4.5
HS 05	215	-	1.2	-	3.7
HS 06	460	-	2.0	-	3.5

Reproducibility:

The reproducibility was assessed by testing the Elecsys CMV IgG assay on the E2010 analyzer at three sites. Imprecision testing was conducted using three replicates in two runs per day for five days consistent with requirements within CLSI EP5-A2 and EP15-A2 with four serum pools and three PreciControl CMV IgG materials. One reagent lot was used for all of the testing at all three sites. The sample panels evaluated included samples close to the cut-off, reactive and non-reactive. All sample panels demonstrated a % CV less than 12%. The results are presented in the table below.

Elecsys CMV IgG Between-Site Reproducibility

			Repeatability		Between-Day		Between-Site		Reproducibility	
Sample	N	Mean COI	SD	% CV	SD	% CV	SD	% CV	SD	% CV
HSP 01 Near cut-off	90	0.858	0.013	1.6	0.004	0.5	0.043	5.0	0.048	5.6
HSP 02 Near cut-off	90	1.146	0.020	1.8	0.000[a]	0.0	0.048	4.2	0.057	5.0
HSP 03 Reactive	90	229.103	2.900	1.3	0.000[a]	0.0	12.284	5.4	13.955	6.1
HSP 04 Non-Reactive	90	951.222	24.119	2.5	18.172	1.9	97.667	10.3	103.456	10.9
PC CMV-IgG_1	90	1.315	0.020	1.5	0.007	0.6	0.084	6.4	0.093	7.0
PC CMV-IgG_2	90	25.940	0.256	1.0	0.000[a]	0.0	1.606	6.2	1.738	6.7
PC CMVIgGNeg	90	1060.067	30.202	2.8	0.000[a]	0.0	106.204	10.0	111.669	10.5

[a] SD of zero due to variance contributed by particular component was below stated significant figure.

b. Linearity/assay reportable range:

Not Applicable

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

Calibration Stability:

Two studies were conducted to evaluate the calibration stability; lot calibration stability and on-board calibration stability.

1. Lot Calibration Stability: The lot calibration stability was determined by comparing the calibration for two kits of the same lot. On day 1, the first reagent kit was opened and calibrated, and samples were measured at days 1 and 28 on one cobas e 411 (analytically equivalent to the Elecsys 2010, the master analyzer for these analytical studies). All samples in both panels were measured in duplicate with one reagent lot in one run per day on days 1 and 28. The acceptance criteria were met for lot calibration stability of 28 days on the cobas e 411.
2. On-board Calibration Stability: On-board calibration stability for the Elecsys CMV IgG test system was tested on one cobas e 411 immunoassay analyzer and on one MODULAR ANALYTICS E170 immunoassay analyzer. One reagent kit was opened and samples were measured on day 1. The same samples were then retested after 8 days (e 411 Panel A and all E170 data) and after 15 days (e 411 Panel B data) with reagent bottles kept at 20±3°C (on-board condition) using the calibration from day 1. Recovery was calculated based on the initial values. All samples were tested in duplicate with one reagent lot on one cobas e 411 and one MODULAR ANALTYICS E170, one run per day (two runs day 1) on two days. The specification was met for on-board calibration stability of 8 days on cobas e 411 and MODULAR ANALYTICS E170 immunoassay analyzers.

Calibrator Stability:

The calibrator stability was evaluated by two studies; calibrator stability at 2-8°C and open vial calibrator stability.

Stability at 2-8°C for 21 days after first opening: Reference and on-test materials were tested in duplicate with one reagent lot in one run per day on one Elecsys 2010 analyzer. The on-test material was opened and kept at 2-8°C for 8 weeks. It was measured at day 0 and at 8 weeks. The on-test recovery was calculated as the signal (counts) of the reference value. The acceptance criteria was a recovery of signal (counts) 90-110 %. The acceptance specification for stability for 8 weeks after first opening was met for an Elecsys CMV IgG Calibrators 1 and 2.

Open Vial Stability: Reference and open vial, on-test materials were tested in duplicate with one reagent lot on one Elecsys 2010 analyzer and on one MODULAR ANALYTICS E170. The on-test material was opened and kept at 25°C for 6 hours on

Elecsys 2010 and for 2 hours on a MODULAR ANALYTICS E170 to simulate on-board conditions. Opened on-test material was also kept at 32°C for 6 hours on Elecsys 2010 and for 2 hours on one MODULAR ANALYTICS E170 to simulate on-board conditions. Every hour each calibrator was tested and the on-test recovery of signal (counts) was calculated as a percent of the reference value. The acceptance criteria were recovery of signal (counts) 90-110 %. The specification was met for open vial stability of 5 hours at 25°C on both the cobas e 411 and of 2 hours on the MODULAR ANALYTICS E170.

Preci-Control Value Assignment:

The Elecsys CMV IgG calibrators and Elecsys PreciControl CMV IgG values are assigned through six independent series of analysis are performed on each instrument. All samples are tested in duplicate. The sample recovery (COI) is calculated as the median of each sample as reference to the target value: PC0: <0.25 COI, PC1: 1.5 COI, PC2: 25 COI

Stability of PreciControl CMV IgG:

To test the stability of the PreciControl CMV IgG the following studies were conducted:

Stability at 2-8°C for 8 weeks after first opening:

Stressed and unstressed samples of PreciControl CMV IgG were tested in duplicate with one reagent lot in one run per day on one Elecsys 2010 analyzer. All samples met the predetermined acceptance specification for signal recovery of (counts) 90-110 %. Elecsys PreciControl CMV IgG levels 1, 2, and 3 are stable up to 8 weeks.

Open Vial Stability:

Samples were tested in singlicate with one reagent lot on one Elecsys 2010 analyzer and on one cobas e 601. The specifications for controls 0, 1, and 2 were met, and PreciControl CMV IgG was stable up to 6 hours on Elecsys 2010 and up to 2 hours on cobas e 601/MODULAR ANALYTICS E170 at 25°C.

Shelf Life Stability:

The samples tested were kept at 2-8°C and tested in duplicate at several time points during and beyond the shelf life time of 21 months. Stability was measured by comparing the measurements of the stressed controls with the measurements of the unstressed controls. The acceptance specifications for Controls 0, 1, and 2 were met. Elecsys PreciControl CMV IgG was stable up to at least 22 months. This supports the claimed shelf life of 21 months.

d. Detection limit:

Not Applicable

e. Analytical specificity:

Interference Study:

To evaluate the effect of elevated levels of hemoglobin, bilirubin, intralipid, biotin, total protein and rheumatoid factor on the CMV IgG assay, six CMV IgG samples (2 negative, 2 near cutoff and 2 positive) were spiked with the potential interferents. All samples were tested in duplicate on the Elecsys 2010 analyzer. Acceptance criteria were recovery of ± 15 %.
The results of the Interference Study are presented below:

Interferent tested	No interference up to
Hemoglobin	< 0.623 mmol/L or < 1.0 g/dL
Bilirubin	< 1129 µmol/L or < 66 mg/dL
Intralipid	< 2000 mg/dL
Biotin	< 409 mmol/L or < 100 ng/mL
Total protein	< 20 g/dL
Rheumatoid factor	< 1600 IU/mL

Cross-reactivity:

A study was conducted to evaluate the Elecsys CMV IgG assay for potential cross-reactivity using samples from individuals with antibodies to various medical conditions. Specimens (n = 249) were tested in duplicate with both the Elecsys CMV IgG assay and the predicate assay. At least 3 CMV IgG negative samples and positive for each potential cross reactant were required to rule out potential cross reactivity. No potential cross-reactivity was detected for samples with antibodies to EBV, HAV, HBV, HCV, HIV, HSV, HTLV, Influenza vaccine, Rubella, Treponema pallidum or Toxoplasma gondii. Potential cross-reactivity with the vector *E. coli* and autoimmune markers could not be ruled out from the study. The potential cross-reactivity was not evaluated for VZV IgG, Measles IgG, Mumps IgG and Parvo-B19 IgG. The following table shows the results of testing the samples for each potential cross reactant with both the Elecsys CMV IgG assay and the predicate device. Due to the high prevalence of CMV IgG antibodies many samples were positive by the Elecsys CMV IgG assay. All of the Elecsys CMV IgG positives were confirmed positive by the predicate device with the exception of one discordant sample for HTLV.

Potential Cross-reactant	No. tested	Elecsys CMV IgG/Predicate Negative/Negative	Elecsys CMV IgG/Predicate Positive/Positive
Autoimmune markers	7	1	6
EBV	15	11	4
E. coli	5	0	5
HAV	12	9	3
HBV	21	4	17

Potential Cross-reactant	No. tested	Elecsys CMV IgG/Predicate Negative/Negative	Elecsys CMV IgG/Predicate Positive/Positive
HCV	35	15	20
HIV	26	3	23
HSV	7	3	4
HTLV[a]	29	11	17
Influenza vaccine	10	3	7
Rubella	15	6	9
Treponema pallidum	58	9	49
Toxoplasma gondii	9	6	3

[a] One discordant result was observed.

f. Assay cut-off:

The assay cut-off value was established from in-house studies by measuring a panel of 931 samples. A Receiver Operator Curve (ROC) analysis was used to optimize sensitivity and specificity. The cut-off value was validated in the clinical study.

g. High Dose Hook-Effect:

Four high-titer CMV IgG samples of human serum were diluted with negative human serum in a dilution series with 11 dilution steps. Each dilution was tested in triplicate with one reagent lot in one run on one Elecsys 2010 analyzer. No hook effect was observed up to 2,500 COI.

2. Comparison studies:

a. Method comparison with predicate device:

See Clinical Study

b. Matrix comparison:

The effect on recovery of analyte in the presence of anticoagulants with the Elecsys CMV IgG was determined on the Elecsys 2010 immunoassay analyzer by comparing values obtained from human samples drawn into serum, lithium-heparin plasma, K2 -EDTA plasma, and K3 -EDTA plasma. A total of 38 serum/plasma pairs at three concentrations (negative, near cut-off, and positive) per sample material were tested with one reagent lot in three runs on one Elecsys 2010 immunoassay analyzer. The reference for all sample types was serum drawn into serum primary tubes (without gel). The acceptance criteria for negative samples was a change in signal less than 0.2 from the serum sample, and for positive samples a recovery of ±20% when compared to serum. The results support the use of the following plasma types: lithium-heparin, K_2-EDTA, and K_3-EDTA. Acceptance specifications are tabulated below.

Plasma matrix	Percent of samples showing differences in recovery relative to serum (COI) in negative specimens		
	< 0.05 (COI)	0.05-0.1 (COI)	> 0.1 (COI)
Li-heparin	42 %	58 %	0 %
K_2-EDTA	84 %	16 %	0 %
K_3-EDTA	53 %	47 %	0 %

Plasma matrix	Percent of samples showing differences in recovery relative to serum (%) in low positive specimens		
	< 10 %	10-20 %	> 20 %
Li-heparin	100 %	0 %	0 %
K_2-EDTA	100 %	0 %	0 %
K_3-EDTA	100 %	0 %	0 %

Plasma matrix	Percent of samples showing differences in recovery relative to serum (%) in high positive specimens		
	< 10 %	10-20 %	> 20 %
Li-heparin	91 %	9 %	0 %
K_2-EDTA	100 %	0 %	0 %
K_3-EDTA	100 %	0 %	0 %

b. Method Comparison Between Analyzers:

The equivalence of results from the Elecsys CMV IgG assay when run on the Elecsys 2010 and MODULAR ANALYTICS E170 immunoassay analyzers was evaluated by a method comparison study. A total of 137 native serum samples were tested in singlicate with one reagent lot on one Elecsys 2010 analyzer and one MODULAR ANALYTICS E170 analyzer. Of these samples, 7 were negative, 119 were positive and 11 were near cut-off. Positive and negative agreements of the results between the two platforms were calculated. The results are summarized in the following table.

		MODULAR ANALYTICS E170		
		Positive	Border	Negative
Elecsys 2010	Positive	119	0	0
	Border	0	10	0
	Negative	0	1	7
	Total	119	11	7
	Total n = 137			

Concordance rates:

Negative agreement - 7/8 = 87.5 % Concordance

Positive agreement - 119/119 = 100 % Concordance

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):

   A multi-center study was conducted in the U.S. to evaluate the ability of the Elecsys CMV IgG assay to detect anti-CMV IgG antibodies. The clinical samples consisted of the following: a total of 605 samples, of which 400 samples were from a population of patients in which a CMV IgG test was ordered and 205 samples were from pregnant women in which a CMV IgG test was ordered.

   Testing of specimens was done at two clinical testing sites and one internal site. The results of these studies are presented in the following tables.

Test Ordered Population n= 605	Comparator device CMV IgG result			
Elecsys CMV IgG result on Elecsys 2010 analyzer	+ (n)	Equivocal (n)	- (n)	Total
+	280	3	12	295
Equivocal	0	0	8	8
-	2	1	299	302
Total	282	4	319	605

Positive agreement	Positive agreement 95 % CI	Negative agreement	Negative agreement 95 % CI
98.94 (280/283)	96.93-99.78	92.86 (299/322)	89.47-95.42

Pregnant Population n=205	Comparator device CMV IgG result			
Elecsys CMV IgG result on Elecsys 2010 analyzer	+ (n)	Equivocal (n)	- (n)	Total
+	84	0	0	24
Equivocal	0	0	0	0
-	2	1	118	121
Total	86	1	118	205

Positive agreement	Positive agreement 95 % CI	Negative agreement	Negative agreement 95 % CI
96.55 (84/87)	90.25-99.28	100.00 (118/118)	96.92-100.00

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   The observed expected values in the clinical study population using the Elecsys CMV IgG assay in the U.S. portion of the study population were as follows:

Elecsys CMV IgG Result					
Age group (years)	Gender	Reactive n (%)	Equivocal n (%)	Non-reactive n (%)	Total (n)
18 to 19	Male	4 (80.00)	0 (0.00)	1 (20.00)	5
	Female	0 (0.00)	0 (0.00)	1 (100.00)	1
20 to 29	Male	18 (40.00)	1 (2.22)	26 (57.78)	45
	Female	19 (48.72)	1 (2.56)	19 (48.72)	39
30 to 39	Male	33 (42.86)	0 (0.00)	44 (57.14)	77
	Female	24 (58.54)	2 (4.88)	15 (36.59)	41
40 to 49	Male	39 (55.71)	1 (1.43)	30 (42.86)	70
	Female	14 (66.67)	0 (0.00)	7 (33.33)	21
50 to 59	Male	34 (56.67)	1 (1.67)	25 (41.67)	60
	Female	10 (66.67)	1 (6.67)	4 (26.67)	15
60 to 69	Male	8 (44.44)	1 (5.56)	9 (50.00)	18
	Female	6 (100.00)	0 (0.00)	0 (0.00)	6
70 to 79	Male	2 (100.00)	0 (0.00)	0 (0.00)	2
	Female	0 (0.00)	0 (0.00)	0 (0.00)	0
All Ages	Male	138 (49.82)	4 (1.44)	135 (48.74)	277
	Female	73 (59.35)	4 (3.25)	46 (37.40)	123
Total		211 (52.75)	8 (2.00)	181 (45.25)	400

N. Proposed Labeling:

The labeling is sufficient and 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.