510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K100433

B. Purpose for Submission:

New device

C. Measurand:

IgM antibodies to Cytomegalovirus (CMV)

D. Type of Test:

Enzyme labeled Chemiluminescent Immunoassay

E. Applicant:

Siemens Healthcare Diagnostics Inc.

F. Proprietary and Established Names:

IMMULITE® 2000 CMV IgM

CMV IgM Controls

G. Regulatory Information:

Product code	Classification	Regulation section	Panel
LKQ- Antibody IgM ,IF, Cytomegalovirus Virus	Class II	866.3175 - Cytomegalovirus Serological Reagents	Microbiology (83)
JIT: Calibrator, secondary	Class II	862.1150 - Calibrator	Clinical Chemistry
JJX: Single (specified) analyte controls (assayed and unassayed)	Class I	862.1660 - Quality Control Material (assayed and unassayed)	Clinical Chemistry

H. Intended Use:

1. Intended use(s):

   For in vitro diagnostic use with IMMULITE® 2000 Systems analyzers — for the qualitative detection of IgM antibodies to cytomegalovirus (CMV) in human

serum or plasma (EDTA or heparinized), as an aid in the diagnosis of current and recent CMV infection in individuals with signs and symptoms of CMV infection or clinical suspicion of CMV infection. This assay is not FDA cleared or approved for use in testing (screening) blood or plasma donors, neonatal screening or for use at a point of care facilities.

Performance characteristics for this assay have not been established in immunocompromised, immunosuppressed individuals, organ transplant individuals.

CMV IgM Controls: CMV IgM Controls are assayed, bi-level controls intended for use with the IMMULITE 2000 CMV IgM assay. They are intended as an aid in monitoring day-to-day assay performance.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   IMMULITE® 2000 Systems analyzers

I. Device Description:

IMMULITE 2000 CMV IgM is a solid-phase, enzyme labeled chemiluminescent three-step immunoassay. The solid phase (bead) is coated with inactivated, purified CMV antigen (strain AD-169 from infected cell lysates). The liquid phase consists of two reagents: 1) polyclonal goat anti-human IgG antibody in buffer and 2) alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-human IgM antibody in buffer. The kit is intended for use with the IMMULITE® 2000 Systems analyzers.

The reagents which are provided in the kit includes the following; CMV IgM Bead Pack 200 beads, CMV IgM Reagent Wedge, Reagent A containing 17.5 mL of a buffer solution with polyclonal goat anti-human IgG, Reagent B containing 11.5 mL of a buffer solution with polyclonal goat anti-human IgG, Reagent C containing 11.5 mL of alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-human IgM antibody (in buffer), CMV IgM Adjustor, one vial of lyophilized human serum with IgM reactive to CMV (with preservative) and CMV IgM Controls (positive and negative controls with preservative). The following reagents are required for the test and supplied separately; chemiluminescent substrate, probe wash, probe cleaning kit and disposable reaction tubes.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   VIDAS® CMV IgM assay

2. Predicate 510(k) number(s):
   K933549

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For in vitro diagnostic use with IMMULITE® 2000 Systems analyzers — for the qualitative detection of IgM antibodies to cytomegalovirus (CMV) in human serum or plasma (EDTA or heparinized), as an aid in the diagnosis of current and recent CMV infection in individuals with signs and symptoms of CMV infection or clinical suspicion of CMV infection. This assay is not FDA cleared or approved for use in testing (screening) blood or plasma donors.	The VIDAS® CMV IgM assay is intended for use with a VIDAS® instrument as an automated enzyme-linked fluorescent immunoassay (ELFA) for the qualitative detection of anti-CMV IgM antibodies in human serum. It is intended to be used as an aid in the diagnosis of cytomegalovirus infection. It is not intended for use in testing (screening) blood or plasma donors.
Antigen Specificity	The solid phase (bead) is coated with inactivated, purified CMV antigen (strain AD-169 from infected cell lysates).	The Solid Phase Receptacle is coated with CMV antigen (strain AD169).
Detection	Qualitative	same
Technology	Enzyme immunoassay-Chemiluminescent	Enzyme-linked immunoassay-fluorescent

Differences		
Item	Device	Predicate
Matrices	Serum or plasma (EDTA or heparinized)	Serum

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

Standards:

No standard documents were referenced.

FDA Guidance Documents:

1. Points to Consider for Collection of Data in Support of In-Vitro Device Submissions for 510(k) Clearance OIVD.

2. Points to Consider for Review of Calibration and Quality Control Labeling for In Vitro Diagnostic Devices/Cover Letter dated 3/14/1996 OIVD.

3. Review Criteria for In Vitro Diagnostic Devices for Detection of IGM Antibodies to Viral Agents OIVD DIHD.

4. Guidance for Industry and FDA Staff: Bundling Multiple Devices or Multiple Indications in a Single Submission CDRH.

L. Test Principle:

IMMULITE 2000 CMV IgM is a solid-phase, enzyme-labeled chemiluminescent three-step immunoassay. The solid phase (bead) is coated with inactivated, purified CMV antigen (strain AD-169 from infected cell lysates). The liquid phase consists of two reagents: 1) polyclonal goat anti-human IgG antibody in buffer and 2) alkaline phosphatase (bovine calf intestine) conjugated to polyclonal goat anti-human IgM antibody in buffer.

In the first cycle, the patient sample and polyclonal goat anti-human IgG antibody are incubated together without the bead for 30 minutes. During this time, anti-IgG antibodies block IgG present in the patient's sample.

In the second cycle, the pretreated sample and polyclonal goat anti-human IgG antibody are transferred to the second reaction tube. Anti-IgG antibodies block the remaining IgG from the patient's sample from binding to the CMV antigen on the bead. During this time, CMV IgM in the patient sample binds to CMV antigen on the bead. Unbound sample and reagent are then removed by centrifugal washes.

In the third cycle, the enzyme conjugated polyclonal goat anti-human IgM antibody is added to the second reaction tube. The enzyme conjugate binds to immobilized IgM to form the antibody sandwich complex. The unbound enzyme conjugate is removed by centrifugal washes. Finally, chemiluminescent substrate is added to the reaction tube and the signal is generated in proportion to the bound enzyme.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

In- House Precision Study: Six serum samples and two controls were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates. The results are presented in the table below.

		Within-Run		Total	
	Mean	SD	CV	SD	CV
1	0.07	0.01	14.3%	0.01	14.3%
2	0.14	0.03	21.4%	0.03	21.4%
3	0.90	0.06	6.7%	0.07	7.8%
4	1.06	0.05	4.7%	0.09	8.5%
5	1.31	0.07	5.3%	0.09	6.9%
6	2.33	0.11	4.7%	0.15	6.4%
7	2.36	0.07	3.0%	0.12	5.1%
8	8.65	0.31	3.6%	0.51	5.9%

External Reproducibility Study: a reproducibility study was conducted at two external sites and the Siemens Healthcare Diagnostics in-house laboratory. The study was designed to test serum pools using one kit lot of the IMMULITE® 2000 CMV IgM assay. Reproducibility was evaluated using three serum pools with target S/CO ratios in the high nonreactive, cut-off and medium/high reactive ranges. Two runs were conducted per day over 5 days (not necessarily consecutive) at each site. Each run included 4 replicates of each serum pool tested. Reproducibility was conducted at one site with a different lot than that used at the other two sites. Pooled results across the sites are provided in the following table:

Reproducibility (ratio) Pooled across 3 sites with two lots

Pool n	Mean of Reps	Within-Run		Between-Run		Between-Site		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	0.63	0.03	4.3%	0.02	2.4%	0.04	7.0%	0.05	8.6%
2	1.06	0.06	5.4%	0.06	5.2%	<0.01	0.3%	0.08	7.5%
3	2.09	0.11	5.4%	0.12	5.6%	0.06	2.9%	0.17	8.3%

b. Linearity/assay reportable range: Not Applicable, the test is a qualitative test

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

Traceability, Stability: As there is no internationally recognized standard for CMV IgM assays, standard industry practice was followed in order to establish the concentration of the initial CMV IgM Adjustor (calibrator) and subsequent lots of the adjustor. Stability information is provided and study plans were evaluated.

Expected values (controls, calibrators): The IMMULITE® 2000 CMV IgM is a qualitative assay reporting results as reactive, nonreactive and indeterminate, although the sample to cutoff ratio of a test sample is also reported on the instrument output. Each kit contains a positive and negative control and the expected ranges are provided on lot-specific insert sheets.

Calibrators: Each kit contains one vial of CMV IgM Adjustor. The Adjustor is used to establish the assay cutoff. The cutoff is equal to the average counts per second (mean cps) of the Adjustor from the most recent adjustment, multiplied by a curve parameter assigned to and provided with each CMV IgM kit lot number. The Master Cutoff of the assay was determined from representative samples to achieve optimal performance for the IMMULITE assay versus reference methods. Titration of subsequent Adjustor lots is performed according to in-house procedures that link the new CMV IgM Adjustor lot to the previous lot and ultimately to the Master Cutoff. This is done so that if the same patient sample is tested across different kit lots, the ratio is similar and also the reactive or non-reactive interpretation for a given sample is the same. The Adjustor is prepared by spiking positive serum into a negative serum matrix according to established manufacturing procedures. The calculation itself and the reporting of qualitative results are performed automatically by the IMMULITE® 2000 analyzer. The recommended Adjustment Interval is two weeks, i.e., the user should perform the adjustment process every two weeks the test system is in use, as verified by Siemens in-house performance testing.

d. *Detection limit:*

Not Applicable, the test is a qualitative test

e. *Analytical specificity:*

Cross reactivity:

The specificity of the IMMULITE 2000 CMV IgM assay was evaluated for a number of potential cross reactive agents; antinuclear antibodies (ANA), syphilis, rheumatoid factor (RF), and IgM antibodies to Epstein-Barr virus (EBV), herpes simplex virus (HSV), rubella virus, *Toxoplasma gondii* and varicella zoster virus (VZV). The study was performed using samples determined to be positive for the specific analytes by approved vendors, using

FDA-cleared assays. The results demonstrated potential cross reactivity with RF and the number of samples tested for HSV was not sufficient to evaluate the potential cross reactivity. The results are presented in the table below.

	n Total Tested	*n* Reactive or Indeterminate
ANA	57	0
EBV	32	0
HSV	3	0
RF	34	2
Rubella	35	0
Syphilis	8	0
Toxo	19	0
VZV	9	0

Interference:

Six serum samples, representing nonreactive, near cutoff, and reactive levels of CMV IgM were spiked with various levels of hemoglobin, triglycerides, and bilirubin at known concentrations to simulate various degrees of hemolysis, lipemia and icterus. Both neat and spiked samples were assayed in duplicate with the percent recovery of observed versus expected mean results and change in qualitative interpretation used to determined presence of interference.

Bilirubin: Presence of bilirubin in concentrations up to 200 mg/L has no effect on results, within the precision of the assay.

Hemolysis: Presence of hemoglobin in concentrations up to 522 mg/dL has no effect on results, within the precision of the assay.

Lipemia: Presence of triglycerides in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay.

f. Assay cut-off:

The assay cut-off was evaluated using a total of 102 samples. The samples were assayed with the IMMULITE® CMV IgM assay. The counts (Kcps) reading from the IMMULITE® 2000 analyzer were compared to the results obtained with the predicate device. The data were analyzed by a Receiver Operating Characteristics (ROC) program in order to choose the optimal cutoff for agreement between IMMULITE® CMV IgM and the predicate

device. The selected cutoff was subsequently verified with use of the first lot of Adjustor set at the cutoff and compared to qualitative results from predicate assay. An "indeterminate zone" has been established for the CMV IgM assay (ratio results between 0.9 and <1.1) where the results cannot be confidently reported to be reactive or nonreactive.

2. Comparison studies:

a. Method comparison with predicate device:

The assay was compared to a commercially available assay for CMV IgM (Kit A) on 400 preselected banked samples at the manufacturer's facility. The results are presented below.

	IMMULITE 2000 CMV IgM (L2KCM)		
Kit A	Reactive	Indeterminate	Nonreactive
Positive	57	1	2
Equivocal	3	0	0
Negative	4	9	324

Positive Agreement: 95.0% (57/60, 95% CI: 86.1% – 99.0%)

Negative Agreement: 95.3% (324/340, 95% CI: 92.5% – 97.3%)

b. Matrix comparison:

The IMMULITE® 2000 CMV IgM assay is indicated for use with serum or plasma samples. A matrix comparison study assessed the degree of equivalence among sample types. Forty matched sets of samples that included serum (red top), SST (gel barrier), sodium heparin and EDTA tubes were tested. Ten (10) of the matched sets were further spiked with various levels of CMV IgM positive serum to obtain values with various reactivity of the analyte. Testing was done and regression analysis of data indicated equivalence among sample tube types. The results are summarized below.

Slope Regression	**r**	**Mean(ratio)**
Serum	Not Applicable	0.75
Heparin = 0.98 (serum) – 0.024	0.997	0.79
EDTA = 1.08 (serum) – 0.055	0.988	0.74
SST = 0.99 (serum) – 0.020	0.996	0.77

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):

Prospective Clinical Study:

The performance of the IMMULITE 2000 CMV IgM assay was evaluated using a total of 527 serum samples prospectively collected at four U.S. sites and three commercial suppliers. The samples were collected from individuals in whom CMV IgM test was required from different target enrollment groups. Each sample was tested with the IMMULITE 2000 CMV IgM assay and with a commercially available CMV IgM assay (Kit A) at three US sites, two external sites and one internal site. The results are presented below.

Comparison for Prospective Subjects

	IMMULITE 2000 CMV IgM		
Kit A	Reactive	Indeterminate	Nonreactive
Positive	8	0	2
Equivocal	2	1	7
Negative	6	6	495

Positive Agreement*: 47.1% (8/17, 95% CI: 23.0% – 72.2%)

Negative Agreement: 97.2 % (495/509, 95% CI: 95.4% – 98.5%

*Note: The number of positive samples was not sufficient to evaluate the positive percent agreement with the predicate device.

Retrospective Clinical Study:

Due to the low prevalence of CMV IgM positive individuals the performance of the IMMULITE 2000 CMV IgM assay was evaluated in 109 retrospective samples in which the CMV IgM positive status was determined by an FDA cleared assay. The samples were from a variety of target populations for CMV IgM testing. Each sample was tested with the IMMULITE 2000 CMV IgM

assay and with a commercially available CMV IgM assay (Kit A) at three US sites, two external sites and one internal site. The results are presented below.

Comparison for Retrospective IgM positive Subjects

	IMMULITE 2000 CMV IgM		
Kit A	Reactive	Indeterminate	Nonreactive
Positive	90	0	1
Equivocal	5	1	1
Negative	2	0	9

Positive Agreement: 97.8% (90/92, 95% CI: 92.4% – 99.7%)

Negative Agreement*: 56.3% (9/16, 95% CI: 29.9% – 80.2%)

PNote: The negative percent agreement was determined from the prospective study. The number of negative samples in this study was not sufficient (and not required) to make statistical valid conclusions about the negative percent agreement with the predicate device.

4. Clinical cut-off**:** See Assay Cut-off

5. Expected values/Reference range:

The IMMULITE 2000 CMV IgM assay was used in a study to evaluate the prevalence of CMV IgM antibodies in prospectively enrolled subjects whose samples were sent to a laboratory for routine CMV testing. The distribution of results for prospectively collected samples by gender and age are shown in the following table.

	N	Positive	Equivocal	Negative	Prevalence
Total	527	16	7	504	3.0%
Gender					
Female	333	6	7	320	1.8%
Male	194	10	0	184	5.2%
Age (Years)					
<18	16	0	0	16	0.0%
18-29	193	5	2	186	2.6%
30-39	77	2	1	74	2.6%
40-49	66	3	1	62	4.5%
50-59	88	5	1	82	5.7%
60+	84	1	2	81	1.2%
Unknown	3	0	0	3	0.0%

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.