

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k051122

B. Purpose for Submission:

New device

C. Measurand:

CMV specific MHC tetramer

D. Type of Test:

Flow cytometric assay

E. Applicant:

Beckman Coulter, Inc.

F. Proprietary and Established Names:

iTag MHC Tetramer-CMV

G. Regulatory Information:

1. Regulation section:
21 CFR § 864.5220, Automated differential cell counter
2. Classification:
Class II
3. Product code:
GKZ, Counter, differential cell
4. Panel:
Hematology (81)

H. Intended Use:

1. Intended use(s):
The Beckman Coulter iTag MHC Tetramer CMV assay is for the identification and enumeration of cytomegalovirus (CMV)-specific CD8+ lymphocytes in whole blood by flow cytometry, and for the assessment of CMV-specific immune status and risk of CMV reactivation in immunosuppressed stem cell transplant recipients. The assay is limited to individuals with the following HLA types: A*0101, A*0201, B*0702, B*0801, B*3501.
2. Indication(s) for use:
Same as intended use.
3. Special conditions for use statement(s):
The device is for prescription use only.
4. Special instrument requirements:
Beckman Coulter Inc. (BCI) EPICS-XL using System II software, BCI FC500 using CXP software and Becton Dickinson (BD) FACSCalibur using CellQuest software. BCI TQ Prep/ImmunoPrep for whole blood sample preparation (could use other preparation instruments or use manual preparation).

I. Device Description:

The iTag MHC Tetramer CMV Kit contains the following

- 1) iTag MHC Tetramer Multi-Allele Start-UP – CMV (ready-to-use)

- iTAg MHC Negative Tetramer (peptide sequence with no known specificity in humans)
 - iTAg MHC A*0101 Class I, peptide sequence VTEHDTLLY
 - iTAg MHC A*0201 Class I, peptide sequence NLVPMVATV
 - iTAg MHC B*0702 Class I, peptide sequence TPRVTGGGAM
 - iTAg MHC B*0801 Class I, peptide sequence ELRRKMMYM
 - iTAg MHC B*3501 Class I, peptide sequence IPSINVHHY
- (The HLA molecules have been modified to minimize non-antigen specific CD8 mediated binding.)
- The company also provides Start-Up for 3 alleles selected from five alleles specified above. Individual iTAg MHC Tetramers can also be purchased.

2) iTAg MHC Tetramer T Cell Typing Kit (ready-to-use)

- anti-CD3-PC5 clone UCHT1 (recognizes the 20 kD epsilon chain of the CD3 antigen)
- anti-CD4-PE clone SFCI12T4D11 (recognizes a 62 kD single-chain transmembrane glycoprotein present CD4 antigen)
- anti-CD8-FITC clone SFCI21Thy2D3 (recognizes an epitope on the α chain of the 76 kD dimeric CD8 antigen)

3) iTAg MHC Tetramer Accessory Product Group (ready-to-use)

- iTAg MHC Tetramer Lyse Reagent (use with fixative reagent)
- iTAg MHC Tetramer Fixative Reagent (use with lyse reagent)

4) Flow-Count™ Fluorospheres (ready-to-use)

Reagents required but not provided:

- Flow-Set™ Fluorospheres
- IMMUNO-TROL™ Control Cells

J. Substantial Equivalence Information:

1. Predicate device name(s):
tetraONE System for EPICS XL with CYTO-STAT tetraCHROME
2. Predicate 510(k) number(s):
k990172
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Tetramer reagents CD8-FITC + tetramer-PE + CD3-PC5	tetraONE SYSTEM for EPICS XL with CYTO-STAT tetraCHROME
Intended Use	Allows simultaneous identification and enumeration of lymphocyte subset in whole blood by flow cytometry	Same
Fluorochrome	FITC, PE and PC5	Same except for addition of ECD
Instrumentation	Flow cytometer	Same
Sample type	Whole blood	Same

Differences		
Item	Device	Predicate
Intended use	CMV-specific CD8+ lymphocytes for assessment of CMV-specific immune status and risk of CMV reactivation in immunosuppressed stem cell transplant recipients. The assay is limited to 5 HLA Class I antigens – A*0101, A*0201, B*0702, B*0801, B*3501	Total CD45+, total CD3+, total CD4+ and total CD8+ lymphocytes for T lymphocyte subset determination
Cell type detected	CMV-specific CD8 subset and T cell subsets	Total T cells and T cell subsets
Detection method	Tetramers (complexes of MHC molecules associated with a CMV specific peptide sequence)	Monoclonal antibodies to specific epitopes on T cell subsets
Acquisition time	Longer (rare events)	Standard
Controls	Negative tetramer (peptide not found in human) and IMMUNO-TROL™ Control Cells	IMMUNO-TROL™ Control Cells
Anticoagulant	EDTA	EDTA, ACD and sodium heparin

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

FDA Guidance Document for 510(k) Submission of Lymphocyte Immunophenotyping Monoclonal Antibodies (9/91); CLSI Standard Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (CLSI Guideline EP9-A); CLSI Standard. Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (CLSI Guideline H3-A5); CLSI Flow Cytometry: Quality Assurance and Immunophenotyping of Lymphocytes (CLSI Guideline H42-A) and CLSI Performance of Single Cell Immune Response Assays (CLSI Guideline I/LA26-A).

L. Test Principle:

The test consists of two panels. The first panel, (T Cell Typing Kit) determines the absolute count of T cell subsets per volume of blood. The second panel (MHC Tetramer Multi-Allele Start-UP – CMV) determines the frequency of CMV-specific cytotoxic T cells. Whole blood sample (EDTA) is incubated with the T Cell Typing reagents (anti-CD3, anti-CD4 and anti-CD8) in tube 1 and in tube 2 with the CMV specific MHC Tetramer Multi-Allele reagents (specific tetramers, anti-CD3 and anti-CD8). After incubation and red blood cell lysis, the samples are centrifuged, and supernatant aspirated. The remaining cell pellets are suspended and assayed on a flow cytometer. In tube 1, the total CD3+ count is determined and a subset analysis performed to count the CD3+CD4+ and CD3+CD8+ cells. In tube 2, the tetramer positive cells are determined by subset analysis of the CD3+CD8+ cells. The number of CMV-specific cytotoxic T cells per volume of blood is then determined by combining the results of the two panels.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Testing was performed using the BCI EPICS-XL flow cytometer. Since HLA-A*0201 is the most common allele found in North American population, analytical performance of the assay was determined using this allele and validated on A*0101 and B*0702. In some studies, B*0801 and B*3501 were also tested. The following table summarizes the HLA alleles used in the performance studies:

Analytical Study	HLA-Specificities				
	HLA-A*0201	HLA-A*0101	HLA-B*0702	HLA-B*0801	HLA-B*3501
Specificity (blood cell populations)	✓				
Specificity (non-CMV infectious disease samples EBV+/CMV-)	✓	✓	✓	✓	✓
Specificity (CMV+ samples with allele/tetramer mismatched)	✓	✓	✓	✓	✓
Linear range	✓	✓	✓		
Accuracy	✓	✓	✓	✓	✓
Recovery	✓	✓	✓		
Sensitivity	✓	✓	✓		
Intra-lab reproducibility	✓	✓	✓	✓	✓
Inter-lab reproducibility	✓	✓	✓		
Specimen collection and handling	✓	✓	✓		
Expected reference range	✓	✓	✓	✓	✓
Flow cytometer instrument comparison	✓	✓	✓		✓

a. Precision/Reproducibility:

Intra-lab reproducibility was performed by three operators for the A*0101, A*0201 and B*0702 CMV tetramers using 3 BCI EPICS-XL flow cytometers. Separate determinations were made for panel 1 and panel 2. Whole blood samples from 3 CMV-seropositive donors per allele were tested at low (< 0.7%), mid (0.7 to 2.0%) and high (> 2.0%) positive tetramer level. Samples were tested in replicates of ten. Reproducibility was further confirmed for B*0801 and B*3501 CMV tetramers by a single operator using one donor per allele at a single CMV tetramer+ percent (a mid level for B*0801 and low level for B*3501) and samples were also tested in replicates of ten.

Allele	Level	Operator 1			Operator 2			Operator 3		
		Tetramer+ cells/μL	SD	%CV	Tetramer+ cells/μL	SD	%CV	Tetramer+ cells/μL	SD	%CV
A*0101	Low	2.81	0.17	6.0	2.41	0.10	4.3	2.16	0.10	4.5
	Mid	4.60	0.22	4.8	4.52	0.27	6.0	4.81	0.15	3.2
	High	30.65	0.39	1.3	29.26	0.96	3.3	28.49	0.72	2.5
A*0201	Low	2.06	0.14	6.8	1.79	0.10	5.6	1.83	0.12	6.4
	Mid	8.62	0.41	4.7	8.02	0.33	4.1	7.54	0.22	2.9
	High	14.30	0.44	3.1	12.26	0.35	2.8	13.15	0.42	3.2
B*0702	Low	2.38	0.16	6.6	2.69	0.18	6.7	2.44	0.19	8.0
	Mid	4.76	0.24	5.0	4.10	0.21	5.0	4.52	0.36	7.9
	High	24.54	0.78	3.2	22.67	0.64	2.8	22.10	0.42	1.9
B*0801	Mid	3.81	0.61	16.1						
B*3501	Low	1.23	0.18	14.4						

Allele	Level	Operator 1			Operator 2			Operator 3		
		% Tetramer+	SD	%CV	% Tetramer+	SD	%CV	% Tetramer+	SD	%CV
A*0101	Low	0.92	0.05	5.9	0.87	0.04	4.3	0.84	0.04	4.4
	Mid	1.12	0.05	4.8	1.13	0.07	6.0	1.10	0.04	3.2
	High	5.15	0.07	1.3	5.36	0.18	3.3	5.11	0.13	2.5
A*0201	Low	0.53	0.04	7.1	0.47	0.03	5.6	0.50	0.03	6.3
	Mid	2.13	0.10	4.7	2.03	0.08	4.0	1.96	0.06	2.9
	High	3.47	0.11	3.1	3.50	0.10	2.8	3.44	0.11	3.2
B*0702	Low	0.77	0.05	6.6	0.88	0.06	6.7	0.71	0.05	7.7
	Mid	1.14	0.06	5.1	1.14	0.06	5.0	1.20	0.09	7.8
	High	5.99	0.19	3.2	5.79	0.16	2.8	5.70	0.11	1.9
B*0801	Mid	0.78	0.13	16.3						
B*3501	Low	0.25	0.04	14.5						

Inter-lab reproducibility was performed at three sites for the A*0101, A*0201 and B*0702 CMV tetramers using 3 BCI EPICS-XL flow cytometers by 3 operators. The 3 sites were within the BCI facility. Separate determinations were made for panel 1 and panel 2. Whole blood samples from 3 CMV-seropositive donors per allele were tested at low (< 0.7%), mid (0.7 to 2.0%) and high (> 2.0%) positive tetramer level. Samples were tested in replicates of ten.

Allele	Level	Tetramer+ cells/ μ L	SD	%CV	% Tetramer+	SD	%CV	CD3+CD8+ cells/ μ L	SD	%CV
A*0101	Low	2.46	0.33	13.3	0.88	0.04	4.6	281	24.3	8.7
	Mid	4.64	0.15	3.2	1.12	0.02	1.4	416	19	4.6
	High	29.47	1.09	3.7	5.21	0.13	2.6	566	25.5	4.5
A*0201	Low	1.89	0.15	7.7	0.50	0.03	6.0	378	9	2.4
	Mid	8.06	0.54	6.7	2.04	0.09	4.2	395	10.5	2.7
	High	13.27	0.98	7.4	3.47	0.03	0.9	382	29.5	7.7
B*0702	Low	2.50	0.16	6.6	0.79	0.09	11.0	321	21.7	6.8
	Mid	4.46	0.33	7.5	1.16	0.03	3.0	384	28.4	7.4
	High	23.10	1.28	5.5	5.83	0.15	2.5	396	11.9	3.0

Additional inter-lab reproducibility was performed at BCI and two external sites (one US and one Canadian) with whole blood samples from 11 CMV-seropositive donors. External Site A had one operator using BD FACSCalibur flow cytometer. Both External Site B and the BCI site had two operators using BCI EPICS-XL and FC500 flow cytometers. Whole blood was collected and tested with 24-72 hours of venipuncture. Samples included both low and mid concentrations of CMV tetramer+ cells. Samples were assayed in duplicate but only the first replicate was used for the analysis to reflect the normal clinical use of the device. Twenty-one results were generated (4 A*0101, 11 A*0201 and 6 B*0702).

Sample	Tetramer+ cells/ μ L	SD	%CV	% Tetramer+	SD	%CV	CD3+CD8+ cells/ μ L	SD	%CV
1	0.68	0.14	19.9	0.23	0.05	20.8	299	34.0	11.4
2	0.79	0.05	6.8	0.42	0.05	12.5	189	11.4	6.1
3	0.79	0.07	8.6	0.48	0.05	11.1	164	10.9	6.7
4	0.87	0.22	25.8	0.17	0.06	12.5	496	63.4	12.8
5	1.00	0.22	21.7	0.48	0.06	12.9	209	21.4	10.2
6	1.73	0.29	17.1	0.46	0.07	15.6	379	68.4	18.1

Sample	Tetramer+ cells/μL	SD	%CV	% Tetramer+	SD	%CV	CD3+CD8+ cells/μL	SD	%CV
7	1.52	0.21	13.7	0.66	0.07	11.2	231	28.6	12.3
8	1.84	0.18	10.0	0.76	0.04	5.0	241	15.5	6.4
9	2.33	0.26	11.3	0.82	0.05	6.4	284	37.1	13.1
10	2.76	0.70	25.2	0.80	0.16	20.6	344	26.4	
11	3.01	0.33	11.1	0.90	0.12	12.9	337	11.6	3.4
12	2.97	0.66	22.3	0.93	0.12	13.3	315	31.7	10.1
13	4.39	1.30	29.6	1.26	0.07	5.9	351	118.1	33.7
14	3.97	1.03	26.1	1.46	0.30	20.9	269	18.5	6.9
15	4.72	0.75	15.8	1.37	0.26	18.9	347	25.2	7.3
16	5.48	0.88	16.1	1.63	0.13	7.8	336	35.8	10.6
17	6.06	0.73	12.1	1.41	0.07	4.7	429	43.2	10.1
18	6.24	0.50	8.1	1.23	0.03	2.6	507	41.5	8.2
19	6.46	0.55	8.5	1.34	0.16	12.2	485	27.9	5.8
20	8.08	1.51	18.7	1.55	0.35	22.8	528	27.4	5.2
21	8.95	0.63	7.1	3.14	0.14	4.4	285	17.4	6.1

The %CV for intra-lab reproducibility ranged between 1.3% and 16.3%. The %CV for inter-lab ranged from 0.9% to 13.3% when performed at BCI facilities and from 2.6% to 29.6% when performed at BCI and two external sites.

b. Linearity/assay reportable range:

Linearity - The assay linear range was determined using whole blood samples from three CMV-seronegative donors spiked with CMV peptide-stimulated T cells. The spiked whole blood samples were serially diluted to 14 concentrations across the assay range. Samples were tested in duplicate. Results of linearity study are shown in table.

Dilutions	N	A*0101-CMV+		A*0201-CMV+		B*0702-CMV+	
		Tetramer+ cells/μL expected	Tetramer+ cells/μL actual	Tetramer+ cells/μL expected	Tetramer+ cells/μL actual	Tetramer+ cells/μL expected	Tetramer+ cells/μL actual
Neat	2	310.1	314.4	115.99	119.06	234.00	236.28
1:2.1	2	148.0	169.4	57.99	60.62	117.00	107.23
1:4.5	2	69.6	64.4	29.00	30.05	58.50	46.37
1:9.6	2	32.8	33.7	14.50	16.00	29.25	24.43
1:20.4	2	15.4	14.7	7.25	8.34	14.63	11.22
1:43.3	2	7.3	6.7	3.62	3.93	7.31	5.44
1:92	2	3.4	3.9	1.81	2.33	3.66	2.58
1:184	2	1.7	1.6	0.91	1.33	1.83	1.51
1:368	2	0.9	0.9	0.45	0.81	0.91	0.57
1:736	2	0.4	0.2	0.23	0.35	0.46	0.28
1:1473	2	0.2	0.2	0.11	0.21	0.23	0.13
1:2946	2	0.1	0.1	0.06	0.14	0.11	0.05
1:5892	2	0.1	0.0	0.03	0.03	0.06	0.03
1:11785	2	0.0	0.0	0.01	0.03	0.03	0.03

Deming regression analyses are summarized in following table:

	Regression parameter	%Tetramers+	Tetramer+ cells/μL	
		N=2	N=2	95% CI
A*0101	R value	0.9891	0.998	0.993 to 0.999
	Slope	1.0640	1.04	0.997 to 1.08
	Intercept	0.6423	0.18	-1.59 to 1.87

	Regression parameter	%Tetramers+	Tetramer+ cells/ μ L	
		N=2	N=2	95% CI
A*0201	R value	0.9891	0.999	0.999 to 1.00
	Slope	1.0498	1.03	1.02 to 1.03
	Intercept	1.0183	0.35	0.23 to 0.47
B*0702	R value	0.9962	0.998	0.993 to 0.999
	Slope	1.0322	0.99	0.96 to 1.03
	Intercept	0.4502	-2.06	-3.37 to -0.81

Results showed acceptable correlation between the expected and actual values for absolute counts of CMV tetramer positive cells. Depending on the sample tested, the upper limit of the linear range for the tetramer-positive cells varied by allele and ranges from 119 cells/ μ L (A*0201) to >300 cells/ μ L (A*0101).

Recovery – This study was performed according to FDA 510(k) Immunophenotyping Guidance. Whole blood sample from one CMV-seronegative donor per allele (A*0101, A*0201 and B*0702) was spiked with blood from a CMV-seropositive HLA matched donor to 3 tetramer levels (low, mid and high). Separate determinations were made for panel 1 and panel 2 and samples were tested in replicates of five. Results for tetramer+ absolute counts and percents are shown below.

Allele	Spiked Level	N	Expected Tetramer+ cells/ μ L	Observed Tetramer + cells/ μ L	SD	%CV	% Recovery	Mean Recovery
A*0101	Low	5	3.14	3.37	0.2	5.81	107	102
	Mid	5	9.23	9.26	0.17	1.8	100	
	High	5	15.91	15.92	0.37	2.29	100	
A*0201	Low	5	0.52	0.43	0.08	17.92	83	84
	Mid	5	1.54	1.29	0.06	4.4	84	
	High	5	2.46	2.13	0.07	3.31	86	
B*0702	Low	5	2.55	2.49	0.24	9.32	98	97
	Mid	5	7.83	7.70	0.19	2.38	98	
	High	5	12.14	11.68	0.3	2.5	96	

Allele	Spiked Level	N	Expected %Tetramer+	Observed %Tetramer +	SD	%CV	% Recovery	Mean Recovery
A*0101	Low	5	1.29	1.40	0.08	5.71	109	104
	Mid	5	2.92	2.96	0.06	1.87	101	
	High	5	3.92	3.95	0.09	2.24	101	
A*0201	Low	5	0.12	0.10	0.02	18.25	83	87
	Mid	5	0.39	0.34	0.02	4.86	88	
	High	5	0.73	0.66	0.02	3.63	90	
B*0702	Low	5	0.63	0.62	0.06	8.91	99	99
	Mid	5	1.86	1.86	0.04	2.4	100	
	High	5	3.07	3.01	0.08	2.54	98	

The overall percent recovery across the three tetramers was 96%.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No reference material and method available.
- d. *Detection limit:*

Functional assay sensitivity is defined as the lowest concentration where the CV is $\leq 20\%$. Results generated in the linear range study were used for the

calculation. Serial dilutions for B*0702 were repeated because results were inconsistent to previous determinations. Functional sensitivity was determined to be 1.0 cell/ μ L for absolute counts and 0.2% for percent of tetramer positive CD8+ cells (see table below).

Allele	% Tetramer+	Tetramer+ cells/ μ L
	N=2	N=2
A*0101	0.16	0.87
A*0201	0.19	0.35
B*0702	0.44	1.51
B*0702 (repeat)	0.16	0.64
Mean B*0702	0.30	1.08
Overall Mean	0.22	0.77
Overall SD	0.07	0.37

e. *Analytical specificity:*

Interference by other cell types - The potential interference of monocytes, granulocytes, platelets and red blood cells was determined for the A*0201 tetramer according to the FDA 510(k) Immunophenotyping Guidance. Whole blood samples from three CMV-seropositive apparently healthy donors with low, moderate and high % tetramers were used and were spiked with each test cell population. For each test cell population, three different concentrations of spiked cell (1x, 2x or 3x the normal cell count) were tested. Monocytes, granulocytes and platelets were tested in replicates of three and red blood cells in replicates of five. There was no significant interference from the tested cell populations at levels up to 900 cells/ μ L (equivalent to 3x normal level) for monocytes (median percent recovery 105% ranged from 93% to 108%); 5000 cells/ μ L (2x level) for granulocytes (median percent recovery 100% ranged 92% to 108%), 3.2×10^5 cells/ μ L (2x level) for platelets (median percent recovery 95% ranged from 88% to 96%) and 1.31×10^7 cells/ μ L (3x level) for red blood cells (median recovery 104% ranged from 104% to 112%). Results are summarized below:

Cell Type	Spiked cell conc.	Low % tetramer				Mid % tetramer				High % tetramer			
		Mean	SD	%CV	% Recovery	Mean	SD	%CV	% Recovery	Mean	SD	%CV	% Recovery
Monocytes	1x	0.13	0	0	100	1.57	0.02	1.3	100	1.78	0.06	3.6	100
	2x	0.14	0.03	18.3	110	1.48	0.07	4.4	94	1.85	0.12	6.7	104
	3x	0.14	0.03	21.4	108	1.46	0.04	2.8	93	1.86	0.01	0.7	105
Granulocytes	1x	0.13	0	0	100	1.57	0.02	1.3	100	1.78	0.06	3.6	100
	2x	0.13	0.01	6.3	100	1.45	0.06	4.1	92	1.92	0.13	6.6	108
	3x	0.12	0.02	13.8	95	1.20	0.03	2.8	76	1.75	0.11	6.4	98
Platelets	1x	0.13	0	0	100	1.57	0.02	1.3	100	1.78	0.06	3.6	100
	2x	0.12	0	3.8	96	1.37	0.11	7.9	88	1.7	0.07	4.1	95
	3x	0.12	0.02	13.8	95	1.32	0.05	4.1	84	1.62	0.03	1.8	91
Red cells	1x	0.18	0.01	8.13	100	0.64	0.05	7.83	100	1.86	0.05	2.46	100
	2x	0.20	0.02	12.33	107	0.74	0.07	10.08	115	1.93	0.11	5.5	103
	3x	0.21	0.01	5.83	112	0.67	0.03	5.17	104	2.01	0.03	1.53	108

Cross-reactivity with EBV – Whole blood samples from 14 EBV-seropositive, CMV-seronegative, apparently healthy honors with HLA specificities of were

HLA A*0101, A*0201, B*0702, B*0801 and B*3501 tested. Samples were tested in duplicate. There were a total of 22 tetramer results and no cross-reactivity was observed. All results were below the lower assay detection limit for absolute count (≤ 1.0 cells/ μ L) and percent of positive cells ($\leq 0.2\%$) (see table below).

# Tetramer results = 22	CMV Tetramer+	
	Cells/ μ L	Percent (%)
Median	0.03	0.005
Mean \pm SD	0.09 ± 0.16	0.018 ± 0.031
Range	0 to 0.64	0 to 0.130
Upper 95 th Percentile	0.48	0.074

Mismatched donor HLA allele and tetramer combinations – Whole blood samples from five CMV-seropositive apparently healthy donors were tested using mismatched tetramers for each donor. HLA alleles of the donors included A*0101, A*0201, B*0702, B*0801 and B*3501. Samples were tested in duplicate. Of the 19 combinations, no cross-reactivity was observed (see results below). All results were below the lower assay detection limit for absolute count (≤ 1.0 cells/ μ L) and percent of positive cells ($\leq 0.2\%$).

# Tetramer results = 19	CMV Tetramer+	
	Cells/ μ L	Percent (%)
Median	0.03	0.010
Mean \pm SD	0.09 ± 0.13	0.016 ± 0.021
Range	0.01 to 0.56	0 to 0.085
Upper 95 th Percentile	0.32	0.055

f. *Assay cut-off:*
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

Comparison of T cell subsets – T cell subset results generated using T Cell Typing Kit with the new device were compared to results of the predicate assay Beckman Coulter tetraONE/tetraCHROME assay according to CLSI Guideline EP9-A. Whole blood samples from 76 healthy donors (equal number of CMV-seropositive and seronegative) were tested. Samples were tested in duplicate. Results were analyzed by Deming regression for absolute counts of CD3+CD8+ and CD3+CD4+ and summarized below:

T subset	Slope	95% CI	Intercept	95% CI	R
CD3+CD8+	1.12	1.06 to 1.18	-15.19	-40.78 to 8.82	0.97
CD3+CD4+	1.08	1.03 to 1.14	0.76	-42.23 to 41.47	0.98

In addition to comparing to the predicate, % recovery for CD3+CD8+ subset detected by Panel 1 was compared to Panel 2 of the new device.

Comparison	Slope	95% CI	Intercept	95% CI	R
New vs. predicate	1.02	0.99 to 1.05	-0.13	-1.24 to 0.93	0.99
Panel 1 vs. Panel 2	1.00	0.97 to 1.03	-0.21	-1.18 to 0.73	0.99

Instrument comparison:

BD FACSCalibur flow cytometer vs. BCI EPICS-XL flow cytometer – Two separate instruments from each manufacturer were tested at a single site with the A*0101, A*0201, B*0702 and B*3501 CMV tetramers. The same gating scheme and protocol were used. Whole blood samples from 16 CMV-seropositive donors were tested in duplicate. These subjects had CMV tetramer+ values within the expected reference range. Deming regression analysis for tetramer+ absolute counts gave an equation of $y = 0.9697x - 0.3255$. The 95% CI for slope and intercept were (0.9383 to 1.0021) and (-0.6315 to -0.0291) respectively. The correlation coefficient (r) was 0.9978.

Deming regression analysis for % tetramer-positive CD8 cells gave an equation of $y = 0.9800x - 0.0152$. The 95% CI for slope and intercept were (0.9514 to 1.0094) and (-0.0744 to 0.0424) respectively. The correlation coefficient (r) was 0.9982.

Deming regression analysis for absolute CD3+CD8+ counts gave an equation of $y = 0.9520x + 1.3572$. The 95% CI for slope and intercept were (0.9075 to 0.9986) and (-16.4046 to 18.3298) respectively. The correlation coefficient (r) was 0.9954. Deming regression analysis for % CD3+CD8+ cells gave an equation of $y = 1.0061x - 0.5339$. The 95% CI for slope and intercept were (0.9602 to 1.0541) and (-2.1255 to 0.9848) respectively. The correlation coefficient (r) was 0.9956.

The BCI EPICS-XL flow cytometer was also compared to the BCI FC500 flow cytometer with HLA A*0101, A*0201 and B*0702 CMV tetramers. One instrument was used for each flow cytometer and all testing were performed at a single site. Whole blood samples from seven apparently healthy CMV-seronegative and 14 CMV-seropositive donors were tested in duplicate with a total of 53 tetramer results. Deming regression analyses for tetramer absolute counts yielded $y = 0.9812x + 0.1527$ ($r = 0.9921$) with 95% CI for slope and intercept of (0.9472 to 1.0165) and (-0.0009 to 0.3011) respectively. Deming regression analysis for % tetramer-positive CD8 cells yielded $y = 1.0426x - 0.0448$ ($r = 0.995$) with 95% CI for slope and intercept of (1.0138 to 1.0723) and (-0.0773 to -0.0133) respectively.

The imprecision for the CMV tetramer+ absolute counts was comparable between instruments with average %CV < 10% for all samples tested.

b. Matrix comparison:

Only EDTA whole blood was used in this device.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Not applicable.

b. *Other clinical supportive data (when a is not applicable):*

Risk assessment

A retrospective feasibility study was performed on 18 CMV-seropositive stem cell transplant (SCT) recipients who were monitored for recovery of CMV-specific CTLs during the first 12 months after SCT. HLA-A2 tetramers were used to quantify CMV-specific CD8⁺ T cells by flow cytometry. The kinetics of regeneration of these CD8⁺ T cells was determined by enumeration of the tetramer⁺ CD8⁺ cells at 2, 3, 6, 9 and 12 months post SCT and CMV pp65 antigenemia was monitored weekly from day 0 to day 150 and at longer intervals thereafter. Serious post transplant outcomes were defined as multiple (≥ 2) episodes of high viral reactivation requiring antiviral therapy, ≥ 4 weeks on ganciclovir or foscarnet, CMVD, or transplant-related mortality. Relative risk was analyzed by maximum tetramer levels in the first 100 days post transplant. Results of this study showed patients with < 2 cells/ μ L were 4.4 and 1.8 times more likely to have recurrent CMV reactivation and CMVD respectively.

Based on these findings, a prospective feasibility study was conducted. The prospective study consisted of 15 allogeneic SCT transplant patients who were followed up to 664 days post transplant for recurrence of CMV and determination of number of tetramer-binding CMV-specific CD8⁺ T cells. Tetramers used included HLA A80101, A*0201, A*2401, B*0702, B*0801 and B*3501. Results showed nine patients had recovered tetramer-binding CMV-specific CD8⁺ T cells to levels ≥ 2 cells/ μ L (Group 1) in the first 2 to 3 months post transplant and eight of the nine patients (89%) did not develop recurrent CMV infections. Of the remaining six patients had < 2 cells/ μ L of CMV-specific CD8⁺ T cells (Group 2), three (50%) had recurrent CMV infections (see table below).

		Recurrent Viremia		
		Yes	No	Total
CMV-Specific CD8 ⁺ T cells	≥ 2 cells/ μ L	1	8	9
	< 2 cells/ μ L	3	3	6
	Total	3	11	15

Study results, therefore, confirmed the initial results, i.e. patients with < 2 CMV-specific CD8⁺ T cells/ μ L were 4.5 times more likely to have recurrent CMV complications.

Since the feasibility studies used a prototype tetramer assay, a comparison study with 24 samples on the prototype and the final assay was performed. Regression analysis gave $y = 1.04x + 2.2$ with $r = 0.9899$.

The prospective pivotal study was conducted at three clinical sites, two in the US and one in Europe. Sample size calculations for the pivotal trial were

based on the prospective feasibility study (see table above). Using Fisher's exact test of equal proportions (test significance level of 0.05, power of 80% and 1-sided), a minimum of 40 subjects was required with 20 subjects in each group.

For the pivotal study, 42 allogeneic stem cell transplant patients were enrolled with at least 6 months of follow-up from three clinical sites. The first patient was enrolled in May 2004 and follow-up through April 2005. A total of 54 subjects were enrolled. Twelve subjects were excluded from the excluded. The table below listed the subjects enrolled, included and excluded from the analysis.

# of Subjects	Site 1	Site 2	Site 3	Total
Enrolled	10	20	24	54
Included	9 (21%)	13 (31%)	20 (48%)	42
Excluded	1	7	4	12
Reasons for exclusion				
Ineligible*	0	1	0	1
HLA-A24 allele only**	1	0	2	3
Early non-CMV related death with 1-2 tetramer results	0	2	0	2
<150 days viral testing insufficient to determine if recurrent viremia occurred	0	2	2	4
Lost to follow-up	0	2	0	2

*CMV negative recipient

** HLA-A24 tetramer not in study

All CMV tetramer tests were performed at the laboratories in the clinical sites following standard protocol. Samples were collected bi-weekly from 28 days post-transplant. The laboratories were trained and qualified using known CMV tetramer samples. The study endpoint was recurrent or persistent CMV infection or CMV Disease (CMVD) and was determined by standard methods used by the study clinical sites for monitoring. The methods used were quantitative CMV DNA PCR, pp65 antigenemia and shell vial culture assay. CMV DNA PCR was used as a secondary assay for pp65 antigenemia and shell vial culture for leukopenic patients or when the sample could not be tested within 24 hours or to verify the shell vial culture result. The following criteria were used to define the patient's CMV status: 1) CMV infection if the CMV copy number was greater than an established threshold used for implementation of anti-viral therapy (ranged from > 500 to > 10,000 copies/mL of plasma depending on the clinical site), or ≥ 2 positive cells per 200,000 PMNs or positive culture, 2) recurrent CMV infection was defined as two or more episodes separated by negative CMV test results, 3) persistent CMV infection was defined as positive results for four or more weeks and 4) CMVD was defined as biopsy proven organ involvement or CMV in bronchoalveolar lavage fluid. The study analyzed recurrent or persistent CMV infection or CMVD occurring after the rise of CMV-specific CD8+ T cells to determine whether these cells were protective against CMV infection.

The maximum response of any individual allele/tetramer in an individual patient was used in the analysis. All subjects meeting the inclusion criteria with 3 or more blood samples or early death with CMV complications were included in the analysis. Statistical analyses were performed using SAS System software. Relative risk of recurrent or persistent CMV infection or CMVD was calculated. Predictive power of possible risk factors was also assessed by univariate and multivariate logistic models.

The 42 stem cell transplant recipients consisted of 18 males (43%) and 24 females (57%). The median age of the subjects were 48y (mean \pm SD = 49y \pm 11y) ranged from 19y to 65y. Thirty-eight were Caucasians, 2 Indians, 1 Asian and 1 not specified. Sixty-four percent (27/42) of the subjects were HLA A*0201, 40% (17/42) A*0101, 36% (15/42) B*0801, 19% (8/42) B*0702 and 7% (3/42) B*3501. The tetramer percents and absolute counts for the serial blood samples over time for all alleles for all subjects are summarized in table below.

	CD3+CD8+ CMV Tetramer+ (%)	CD3+CD8+ CMV Tetramer+ (cells/ μ L)
Number of Tetramer Results	638	635*
Median	1.43	3.2
Mean \pm SD	4.02 \pm 6.12	20.0 \pm 43.2
Range	0.00-36.53	0.00-274.9
Upper 95 th percentile	15.01	108.7

* CD8+ absolute counts unavailable for 3 subjects

The correlation between absolute counts and percentages of CMV tetramer+CD8+ T cell was also analyzed by regression analysis and shown to be $y = 5.58x - 2.51$ with a correlation coefficient of 0.79. Due to the highly variable total CD8+ T cell counts in SCT patients, the absolute count appeared to be a more accurate measure of CMV immune status and should be used for assessing risk and management of patient post SCT.

Tetramer results of all patients were evaluated 50 to 100 days post-transplant. Day 65 was determined to be the best time point to assess recovery of CMV response since there were sufficient number of blood draws per patient for the analysis (100% of patients had ≥ 2 samples and 83% had ≥ 3) and time points after Day 65 could not distinguish patients with “rapid recovery” from those with “delayed recovery”. The tetramer threshold of 7 cells/ μ L for predicting risk was determined by evaluation of a range of CMV tetramer+ T cell counts in “rapid recovery” patients (see table below).

Tetramer+ Threshold (cells/ μ L)	% Rapid Recovery Patients with Recurrent or Persistent CMV Infection or CMVD	Fisher's Exact p value	Relative Risk (Confidence Limits)
2	25.8	0.09	2.1 (0.9-4.7)
3	24.1	0.06	2.2 (1.0-5.1)
4	16.7	0.01*	3.3 (1.2-8.9)

Tetramer+ Threshold (cells/ μ L)	% Rapid Recovery Patients with Recurrent or Persistent CMV Infection or CMVD	Fisher's Exact p value	Relative Risk (Confidence Limits)
5	16.7	0.01*	3.3 (1.2-8.9)
6	16.7	0.01*	3.3 (1.2-8.9)
7	14.3	0.01*	3.7 (1.2-11.3)
8	17.7	0.07	2.5 (0.8-7.6)

*Statistically significant

Study results summarized in table below show that 21 of the 42 SCT patients had ≥ 7 cells/ μ L between Day 28 and Day 65 post transplant. Only 3 patients (14%) in this group had recurrent or persistent CMV infection or CMVD. The remaining 21 patients had < 7 cells/ μ L and 52% (11/21) developed recurrent or persistent CMV infection or CMVD (see table below).

	Recurrent or Persistent CMV Infection or CMVD		
	No	Yes	Total
Delayed Recovery < 7 cells/ μ L	10	11	21
Rapid Recovery ≥ 7 cells/ μ L	18	3	21
Total	28	14	42

Data also showed that of the 14 patients with recurrent or persistent CMV infection or CMVD, 64% (9/14) had events occurred within the first 100 days post transplant.

The risk of recurrent or persistent CMV infection or CMVD for CMV tetramer+ CD8+ T cells of < 7 cells/ μ L is 52.4% and for CMV tetramer+ CD8+ T cells of ≥ 7 cells/ μ L is 14.3%. Therefore, the relative risk is 3.7 (95% CI 1.2-11.3) for patients with < 7 cells/ μ L CMV tetramer+ CD8+ T cells.

Reproducibility:

Intra-assay imprecision – Each site tested a unique CMV specimen with known reactivity to HLA A*0201 CMV tetramer. The sample was analyzed in triplicate by three operators (Site 1 and 2) and 2 operators (Site 3). Each site used a different flow cytometer – FC500 (Site 1), FACSCalibur (Site 2) and EPICS XL (Site 3). Intra-assay imprecision results for CMV tetramers were within the acceptable limits of $\leq 20\%$ and are shown below. In addition, percents CV of absolute counts of CD3+CD8+ cells were $< 10\%$ ranging from 2.4% to 6.9%.

Site	Operator	System	N	CMV Tetramer+ (%)		CMV Tetramer+ (cells/ μ L)	
				Mean	%CV	Mean	%CV
1	1	FC500	3	3.02	7.3	7.0	4.5
	2			4.60	10.8	13.9	10.8

Site	Operator	System	N	CMV Tetramer+ (%)		CMV Tetramer+ (cells/ μ L)	
				Mean	%CV	Mean	%CV
2	3	FACSCalibur	3	4.66	5.3	18.12	6.9
	1			3.90	17.4	14.7	15.7
	2			2.79	11.4	8.7	11.4
	3			2.99	8.3	9.9	8.3
3	1	EPICS XL	3	4.02	2.11	16.9	4.9
	2			4.47	5.37	18.3	3.1

Inter-laboratory reproducibility – EDTA blood specimens from three donors of known tetramer percents. HLA specificity for the low tetramer activity was HLA A*0201, for mid tetramer activity was HLA A*0101 and for high tetramer activity was HLA B*0702. Samples were stabilized for 7 days and shipped overnight to 5 clinical sites for testing. The clinical sites consisted of the three sites from the intra-assay precision study, BCI and a Canadian laboratory. The specimens were tested within 1-7 days. For each clinical site, assay was tested with one replicate and by one operator. Results were within the acceptable limits of $\leq 20\%$ for CMV tetramer+ results and $\leq 15\%$ for CD3+CD8+ absolute counts.

Clinical performance across sites – CD3+CD8+ CMV tetramer absolute counts from patients with recurrent or persistent CMV infection or CMVD were compared separately from those without. Results showed that patients with recurrent or persistent CMV infection or CMVD had lower tetramer+ cell counts. None of the nine patients in Site 1 developed recurrent or persistent CMV infection or CMVD because the pre-transplant conditioning regimen used was reduced intensity and non-myeloablative which resulted in higher post-transplant white blood cell counts and possibly accounted for faster recovery of CMV immunity. Site 2 and 3 on the other hand used myeloablative regimens.

4. Clinical cut-off:
CMV tetramer+ CD8+ T cells ≥ 7 cells/ μ L at Day 65 post-transplant.
5. Expected values/Reference range:
The expected reference range was established using the A*0101, A*0201, B*0702, B*0801 and B*3501 CMV tetramers on whole blood samples from 36 CMV sero-negative and 36 CMV sero-positive donors. A total of 99 distinct results were obtained, consisting of 57 sero-negative and 42 sero-positive results using the appropriate allele matched tetramers for each donor. Samples were tested in duplicate. Results showed that the reference range for CMV sero-negative subjects was 0 to 0.75 CMV tetramer+ cells/ μ L or 0% to 0.158% CMV tetramer+ percent and for CMV sero-positive subjects the reference range was 0 to 46.57 tetramer+ cells/ μ L or 0.005% to 8.635% CMV tetramer+ percent (see results below).

	CMV sero-negative (57)		CMV sero-positive (42)		Total (99)	
	Tetramer+ absolute counts (cells/μL)	% tetramer+	Tetramer+ absolute counts (cells/μL)	% tetramer+	Tetramer+ absolute counts (cells/μL)	% tetramer+
Mean	0.09	0.026	6.96	1.507	3.00	0.652
SD	0.16	0.03	9.49	1.79	7.02	1.37
Median	0.03	0.01	4.03	0.845	0.12	0.040
Range	0-0.75	0-0.158	0.01-46.57	0.005-8.635	0-46.57	0-8.635
Upper 95 th Percentile	0.19	0.074	24.50	5.40	11.39	2.84

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.