

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

**A. 510(k) Number:**

K153538

**B. Purpose for Submission:**

New Device

**C. Measurand:**

CMV-Specific MHC Dextramer

**D. Type of Test:**

Semi-Quantitative Flow Cytometric Assay

**E. Applicant:**

Immudex ApS

**F. Proprietary and Established Names:**

Dextramer® CMV Kit

**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)

Immunology (82)

## H. Intended Use:

### 1. Intended use:

Dextramer® CMV Kit is a semi-quantitative assay intended for the identification and enumeration of cytomegalovirus (CMV)-specific CD8+ T cells in anticoagulated (Na Heparin) whole blood specimens by flow cytometry.

Dextramer® CMV Kit is indicated for assessment of CMV-specific immune status and risk of CMV reactivation in adult human stem cell transplant patients following immunosuppression and used in conjunction with other laboratory and clinical findings.

The kit cannot be used to measure CMV infection or disease.

The kit is limited to individuals with the following HLA types: A\*0101, A\*0201, B\*0702, B\*0801, B\*3501.

### 2. Indication for use:

Same as Intended Use

### 3. Special conditions for use statement:

For prescription Use only

### 4. Special instrument requirements:

Becton Dickinson FACSCanto II flow cytometer using Diva software

## I. Device Description:

The Dextramer® CMV Kit comprises nine different CMV Dextramers representing seven different alleles as well as three antibodies recognizing CD3, CD4, and CD8:

Dextramer reagents:

- HLA-A\*0101 PE-labeled dextran, Class I, peptide sequence VTEHDTLLY, 25 tests/0.25 ml
- HLA-A\*0201 PE-labeled dextran, Class I, peptide sequence NLVPMVATV, 50 tests/0.50 ml
- HLA-B\*0702 PE-labeled dextran, Class I, peptide sequence TPRVTGGGAM, 25 tests/0.25 ml
- HLA-B\*0801 PE-labeled dextran, Class I, peptide sequence ELRRKMMYM, 25 tests/0.25 ml
- HLA-B\*3501 PE-labeled dextran, Class I, peptide sequence IPSINVHHY, 25 tests/0.25 ml
- Negative control PE-labeled dextran coupled with HLA-B\*801 molecules complexed with nonsense peptide, 150 tests/1.50 ml

Antibodies:

- Anti-CD8/FITC clone SK1 or clone DK25, 2 ml
- Anti-CD3/PerCP clone SK7 or clone UCHT1, 2 ml
- Anti-CD4/PE clone SK3 or clone MT310, 2 ml

Reagents required but not provided:

- FACS Lysing Solution (10X)
- Trucount tubes
- Control Cells

**J. Substantial Equivalence Information:**

1. Predicate device name:  
Beckman Coulter iTag MHC Tetramer CMV Kit
2. Predicate 510(k) number:  
K051122
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	Dextramer CMV Kit	iTag MHC Tetramer CMV Kit
Intended Use	Dextramer® CMV Kit is a semi-quantitative assay intended for the identification and enumeration of cytomegalovirus (CMV)-specific CD8+ T cells in anticoagulated (Na Heparin) whole blood specimens by flow cytometry. Dextramer® CMV Kit is indicated for assessment of CMV-specific immune status and risk of CMV reactivation in adult human stem cell transplant patients following immunosuppression and used in conjunction with other laboratory and clinical findings.	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 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.

Similarities		
Item	Device	Predicate
	The kit cannot be used to measure CMV infection or disease. The kit is limited to individuals with the following HLA types: A*0101, A*0201, B*0702, B*0801, B*3501.	
Instrumentation	Flow Cytometer	same
Sample type	Whole Blood	same
Cell Type detected	CMV-specific CD8+ T cells  Human T lymphocytes	Same  Same
Fluorochromes	Anti-CD8 FITC Anti-CD4 PE	Anti-CD8 FITC Anti CD4 PE
HLA Types (peptide sequence)	HLA-A*0101 (VTEHDTLLY)  HLA-A*0201 (NLVPMVATV)  HLA-B*0702 (TPRVTGGGAM)  HLA-B*0801 (ELRRKMMYM)  HLA-B*3501 (IPSINVHHY)	Same  Same  Same  Same  Same

Differences		
Item	Device	Predicate
Fluorochromes	Anti-CD3 PerCP	Anti-CD3 PC5
Anticoagulant	Na Heparin	EDTA
MHC Multimer backbone	Dextran	Streptavidin
Detection Method	Dextramers	Tetramers (complexes of MHC molecules associated with a CMV specific peptide sequence)

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

1. ISO 14971 Medical Devices-Application of Risk Management to Medical Devices Guidance for Industry;2007
2. CLSI EP09-A3, Method Comparison and Bias Estimation Using Patient Samples; Third Edition; Approved Guideline
3. CLSI H3-A6, Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Sixth Edition
4. CLSI H42-A Flow Cytometry: Quality Assurance and Immunophenotyping of Lymphocytes
5. CLSI I/LA26-A, Performance of Single Cell Immune Response Assays; Second Edition;2013

#### **L. Test Principle:**

The Dextramer CMV Kit test involves a two-step procedure followed by analysis by flow cytometry. Step 1 is the determination of the percentage of CMV-specific CD3+CD8+T cells in the sample (Tube A). A negative control /PE Dextramer is added to Tube B in place of the HLA matching CMV Dextramer. Whole Blood sample (Na Heparin) is incubated with the selected CMV Dextramer matching the HLA-type(S) of the patient in tube A or the negative control in tube B. If a blood sample is analyzed by more than one CMV Dextramer, a separate Tube A for each CMV Dextramer is prepared. Anti-CD8/FITC and anti-CD3/PerCP are added to Tube A and Tube B and then incubated for a second time. After incubation and red blood cell lysis, the samples are centrifuged, washed in PBS, and supernatant is poured off. The remaining cell pellets are suspended in a fixing solution and assayed on a flow cytometer. Step 2 is the determination of the absolute number of CD3+CD8+ T cells in the sample (Tube C). The same whole blood sample is added to a TruCOUNT tube. Anti-CD8/FITC, anti-CD4/PE, and anti-CD3/PerCP are added to the tube and incubated. After incubation and red blood cell lysis, the samples are assayed on a flow cytometer. In Tube A, the percentage of CMV-specific CD3+CD8+ cells determined by subset analysis of the CD3+CD8+ cells. In Tube C, the absolute count of CD3+CD8+ T Cells is calculated. The absolute number of CMV-specific CD3+CD8+ T cells/ $\mu$ L blood is then determined by mathematical calculations.

#### **M. Performance Characteristics:**

**Note: All results below met the manufacturer's pre-specified acceptance criteria.**

##### 1. Analytical performance:

###### *a. Precision/Reproducibility:*

Within-laboratory reproducibility was performed by two operators for the A\*0101, A\*0201, B\*0702, B\*0801, and B\*3501 CMV dextramers using one FACSCanto II flow cytometer. A panel of three whole blood samples per allele were tested at negative ( $<1$  cell/ $\mu$ L), low (1-7 cells/ $\mu$ ), and high ( $\geq 7$  cells/ $\mu$ L) CMV-Specific CD8+ T cells. Samples were tested in replicates of ten per operator with a total of 60 values per allele.

HLA-Type Allele	CMV T-cell Level	N	Operator 1			Operator 2		
			Dextramer cells/ $\mu$ L Range of Replicates	SD	%CV	Dextramer cells/ $\mu$ L Range of Replicates	SD	%CV
A*0101	Neg	10	0.00	0.00	NC	0.00-0.17	0.05	NC
	Low	10	4.44-5.86	0.42	8.60	3.82-5.42	0.48	10.27
	High	10	27.01-30.92	1.14	3.97	26.13-32.72	1.79	6.22
A*0201	Neg	10	0.00	0.00	NC	0.00-0.17	0.06	NC
	Low	10	0.92-1.90	0.26	16.16	1.23-1.94	0.19	12.33
	High	10	8.84-11.21	0.67	6.74	8.05-11.14	0.84	8.85
B*0702	Neg	10	0.00	0.00	NC	0.00-0.09	0.03	NC
	Low	10	3.47-4.20	0.21	5.33	2.97-3.87	0.28	8.30
	High	10	14.81-16.49	0.56	3.61	14.49-16.84	0.92	5.91
B*0801	Neg	10	0.26-0.58	0.09	NC	0.31-0.55	0.08	NC
	Low	10	3.76-5.79	0.58	12.15	4.38-6.06	0.54	10.37
	High	10	7.23-8.74	0.52	6.47	4.61-8.11	1.00	14.49
B*3501	Neg	10	0.00	0.00	NC	0.00-0.03	0.01	NC
	Low	10	2.95-4.43	0.44	11.78	2.64-3.89	0.34	9.62
	High	10	19.11-23.02	1.29	6.06	16.26-20.85	1.20	6.42

HLA Type Allele	CMV Dextramer	CMV T cell Level	N	Mean (cells/ $\mu$ l)	SD (cells/ $\mu$ l)	%CV
A*0101	HLA-A*0101 / VTEHDTLLY / PE	Negative	20	0.02	0.04	NC
		Low	20	4.80	0.49	10
		High	20	28.7	1.50	5
A*0201	HLA-A*0201 / NLVPMVATV / PE	Negative	20	0.03	0.05	NC

HLA Type Allele	CMV Dextramer	CMV T cell Level	N	Mean (cells/ $\mu$ L)	SD (cells/ $\mu$ L)	%CV
		Low	20	1.60	0.23	14
		High	20	10.1	0.93	9
B*0702	HLA-B*0702 / TPRVTGGGAM / PE	Negative	20	0.02	0.03	NC
		Low	20	3.73	0.40	11
		High	20	16.2	1.15	7
B*0801	HLA-B*0801 / ELRRKMMYM / PE	Negative	20	0.43	0.08	NC
		Low	20	5.12	0.61	12
		High	20	7.51	1.00	13
B*3501	HLA-B*3501 / IPSINVHHY / PE	Negative	20	0.00	0.01	NC
		Low	20	3.78	0.44	12
		High	20	21.1	2.27	11

%CV was Not Calculated (NC) for negative samples with CMV-Specific T cells of <1 cell/ $\mu$ L

Site-to-site reproducibility was performed at three clinical laboratory sites for the A\*0101, A\*0201, B\*0702, B\*0801, and B\*3501 CMV dextramers using the FACSCanto II flow cytometers. The study included three different operators using three different flow cytometers with replicates of three performed in one run including samples across negative (<1 cell/ $\mu$ L), low (1-7 cells/ $\mu$ L), and high ( $\geq$  7 cells/ $\mu$ L) CMV Specific CD8+ T cells. Each sample was tested in triplicate using the HLA matched CMV Dextramer for a total of 43 replicates per allele. Results for each site are provided for With-in Run imprecision.

#### Combined results

Sample	N	Mean	Within-Run Site 1		Within-Run Site 2		Within-Run Site 3		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
A*0101 High	9	15.5	1.31	7	0.49	4	1.59	10	2.76	18
A*0101 Low	9	5.31	0.58	10	0.54	13	0.83	13	0.91	17
A*0101 Low	7	5.64	0.58	11	N/A	N/A	0.24	4	0.33	6
A*0101 Low	7	2.44	0.16	6	N/A	N/A	0.31	15	0.27	11

Sample	N	Mean	Within-Run Site 1		Within-Run Site 2		Within-Run Site 3		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
A*0101 Negative	9	0.01	0.04	NC	0.00	NC	0.00	NC	0.02	NC
A*0201 High	9	16.5	1.66	9	1.02	7	0.19	1	1.29	8
A*0201 Low	7	5.62	0.30	5	N/A	N/A	0.33	7	0.55	10
A*0201 Low	9	2.96	0.12	4	0.25	10	0.21	6	0.27	9
A*0201 Negative	9	0.41	0.21	NC	0.05	NC	0.14	NC	0.12	NC
A*0201 Negative	9	0.01	0.01	NC	0.00	NC	0.03	NC	0.01	NC
B*0702 High	8	7.65	0.69	9	0.82	12	0.43	5	0.51	7
B*0702 Low	9	2.63	0.22	10	0.15	6	0.05	2	0.39	15
B*0702 Negative	8	0.00	0.00	NC	0.00	NC	0.00	NC	0.00	NC
B*0801 High	9	9.90	1.02	9	0.40	5	0.95	9	1.789	18
B*0801 Low	7	5.01	0.50	9	N/A	N/A	0.28	5	0.67	13
B*0801 Negative	9	0.00	0.00	NC	0.00	NC	0.00	NC	0.00	NC
B*3501 High	8	7.17	1.20	16	0.30	4	0.16	3	0.47	7
B*3501 Low	9	4.43	0.26	6	0.54	14	0.65	13	0.50	11
B*3501 Negative	9	0.00	0.01	NC	0.00	NC	0.00	NC	0.00	NC

N/A: not applicable; sample not run at site

%CV was Not Calculated (NC) for negative samples with CMV-Specific T cells of <1 cell/ $\mu$ L.

The %CV for within-lab reproducibility ranged between 5% and 14%. The %CV for site-to-site reproducibility ranged from 6% and 18%.

*b. Linearity/assay reportable range:*

The assay linear range was determined using five Na Heparin whole blood samples from stem cell transplant patients representing all five alleles. Serial dilutions of each specimen with CMV seronegative blood specimens were tested in triplicate using a FACSCanto II flow cytometer. Results of linearity study are shown below in tables.

Dilutions	N	A*0101			A*0201		
		Dextramer+ Cells/ $\mu$ L expected	Dextramer+ Cells/ $\mu$ L actual	% Recovery	Dextramer+ Cells/ $\mu$ L actual	Dextramer+ Cells/ $\mu$ L expected	% Recovery
Neat	3	26.33	26.33	100	107.32	107.32	100
1:1.8	3	14.81	14.25	96	60.37	53.29	88
1:3.2	3	8.33	6.89	83	33.96	29.53	87
1:5.7	3	4.69	3.43	73	19.10	16.16	85
1:9.99	3	2.64	2.53	96	10.74	9.64	90
1:17.8	3	1.48	1.13	76	6.04	4.97	82
1:31.6	3	<1	<1	-	3.40	2.99	88
1:56.1	3	<1	<1	-	1.91	1.61	84
1:99.8	3	<1	<1	-	1.08	0.94	87
1:177	3	<1	<1	-	<1	<1	-

Dilutions	N	B*702			B*801		
		Dextramer+ Cells/ $\mu$ L expected	Dextramer+ Cells/ $\mu$ L actual	% Recovery	Dextramer+ Cells/ $\mu$ L actual	Dextramer+ Cells/ $\mu$ L expected	% Recovery
Neat	3	46.95	46.95	100	25.85	25.85	100
1:1.8	3	26.41	27.85	105	14.54	19.49	134
1:3.2	3	14.86	15.91	107	8.18	7.62	93
1:5.7	3	8.36	9.43	113	4.60	4.01	87
1:9.99	3	4.70	5.38	114	2.59	2.48	96
1:17.8	3	2.64	3.12	118	1.46	1.28	88
1:31.6	3	1.49	1.98	133	<1	<1	-
1:56.1	3	<1	<1	-	<1	<1	-
1:99.8	3	<1	<1	-	<1	<1	-
1:177	3	<1	<1	-	<1	<1	-

Dilutions	N	B*3501		
		Dextramer+ Cells/ $\mu$ L expected	Dextramer+ Cells/ $\mu$ L actual	% Recovery
Neat	3	19.19	19.19	100
1:1.40	3	13.71	14.45	105
1:1.96	3	9.79	10.59	108
1:2.74	3	6.99	6.59	94
1:3.84	3	5.00	4.92	98
1:5.38	3	3.57	3.60	101
1:7.53	3	2.55	2.37	93
1:10.54	3	1.82	1.69	93
1:14.76	3	1.30	1.04	80

	r	Slope	95%CI	Intercept	95%CI
A*0101	0.9982	1.02	0.94–1.10	–0.82	–1.90–0.27
A*0201	0.9978	0.98	0.92–1.04	–1.46	–3.99–1.07
B*0702	0.9996	0.99	0.96–1.03	0.84	0.14–1.54
B*0801	0.9794	1.07	0.76–1.38	–0.09	–4.00–3.82
B*3501	0.9982	1.03	0.98–1.09	–0.18	–0.68–0.31

Cell concentrations were tested for linearity up to  $10^7$  cells/ $\mu$ L. The assay shows linearity between 1– $10^7$  cells/ $\mu$ L.

Depending on the sample tested, the upper limit of the linear range (determined by the highest measured concentration for each allele) for the dextramer-positive cells varied by allele and ranges from 110 cells/ $\mu$ L (A\*010) to 19.0 cells/ $\mu$ L for B\*3501.

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

Traceability:

There are currently no international reference standards for flow cytometry available.

Stability:

*Sample Stability:* The Specimen Stability study was performed using blood from four healthy donors collected in heparin tubes with high, low and negative CMV- specific CD8+ T cells. Each sample was analyzed in triplicate using the HLA matched CMV Dextramer from the Dextramer® CMV Kit within 3 hours, 20–22 hours and 44–48 hours post blood draw using a FACSCanto II flow cytometer (Becton Dickinson). CD3/CD4/CD8 low and high cell controls were used as quality controls for the determination of the % and absolute numbers of lymphocyte subsets to ensure that the reagents adhere to the defined criteria during the study. The study supported stability of blood specimens collected in sodium heparin for up to 44–48 hours prior to analysis.

A processed-sample is a specimen that is drawn, prepared, and stained with the protocol for sample preparation before flow cytometric analysis. A processed-sample stability study was performed using four healthy donors collected in Na-heparin tubes representing with high, low and negative CMV- specific CD8+ T cells. Each sample was analyzed in triplicate using the HLA matched CMV Dextramer from the Dextramer® CMV Kit and stored at 2–8°C in the dark prior to flow cytometer acquisition. Flow cytometer acquisition was performed within 3–6 hours and 20–26 hours post staining using a FACSCanto II flow cytometer (Becton Dickinson). CD3/CD4/CD8 low and high cell controls were used to QC the determination of the % and absolute numbers of lymphocyte subsets. Results of the study demonstrated that processed samples may be stored for up to 24 hours prior to analysis by flow cytometry.

*Shelf Life:* Stability testing was performed using only the open vial stability which will represent the open-vial stability as well as the shelf life for the device.

*Open/In use Stability:* A real-time, open-vial stability study was performed using three production lots. The functionality of the Dextramer CMV kit was evaluated using blood samples representing all claimed HLA-types (A\*0101, A\*0201, B\*0702, B\*801, and B\*3501) and demonstrated an open-vial stability of 9 months at 2–8°C. Additional real-time open-vial stability studies are ongoing

*d. Detection limit:*

Limit of Blank (LoB): Fifty one Na heparin blood specimens from CMV seronegative stem cell transplant recipients representing five alleles were included in the study. 100% (51/51) were negative and within 0.00–0.06 cells/μL and thus below the functional assay sensitivity of 1 cell/μL.

Limit of Detection (LoD): Ten Na heparin whole blood specimens from stem cell transplant recipients representing five alleles were included in the study. Serial dilutions of each specimen were tested in triplicate for a total of 48 measurements for each allele. The Limit of Detection (LoD) was determined to be 1 cell/μL.

Limit of Quantitation (LoQ): The Limit of Quantitation (LoQ) (analytical sensitivity) is 1 cell/μL as determined by the lowest concentration of cells (cells/μL) that can be determined with a CV% below 20%.

*e. Analytical specificity:*

98 % (61/62) of CMV seronegative patients have undetectable (<1 cell/μL) CMV-specific T cells ranging from 0.00–0.25 cells/μL.

Interference: Samples from healthy human donors were selected to represent the five alleles and to represent negative, low, and high CMV T-cell response. For each test cell population, three different concentrations of spiked cell were prepared with 1x (unspiked), 2x (spiked), and 3x (spiked) levels of interfering white blood cell and normal, middle, and high level of red blood cells. Each sample was tested in three replicates on the FACSCanto II flow cytometer. There was no significant interference from the tested cell populations equivalent to 2x normal level for monocytes (recovery of 91–114% for 2x ), equivalent to 3x normal level for granulocytes ( recovery of 91–109% for 2x and 100–117% for 3x), equivalent to 3x normal level for platelets (recovery of 87–104% for 2x and 81–110% for 3x), and equivalent to 2x normal level for red blood cells (recovery of 91–109% for middle level and 89–99% for high level).

Cross reactivity with mismatched alleles: No significant cross-reaction was observed when a sample with an allele that was different than the allele being measured was used in the analysis.(mismatching Dextramer CMV reagents). All results from

analysis of four blood samples with CMV-specific T cells with HLA mis-matched CMV dextramers were within 0.00–0.11 cells/ $\mu$ L and thus below the Limit of Detection of 1 cell/ $\mu$ L.

*f. Assay cut-off:*

Not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

The method comparison was performed according to CLSI Guideline EP09-A3, at one site using native samples from the intended use (IU) population and spiked samples on a Becton Dickinson (BD) FACSCanto II flow cytometer. The same set of patient samples was used to perform the testing of CD3+/CD8+ T-cells and CMV specific CD8+ T-cells with the iTAg predicate. A total of 133 samples were tested including CMV-seropositive and seronegative patients for a total of 188 measurements of which 117 have detectable CMV-specific T-cells. For spiked samples, a Na heparin blood sample containing CMV-specific CD8+ T-cells of interest was spiked into multiple different CMV-negative Na heparin blood samples in order to generate 97 unique samples around the cutoff. Spiked samples were used because native samples from the intended use population with CMV-specific T cells around the cut-off are rare.

The total number of patient samples for each of the five claimed alleles as well as the subset with detectable CMV-specific T-cells in parenthesis is listed in the table below:

CMV Dextramer	Native IU Samples (number with values > 1 cell/ $\mu$ L)	Spiked Samples (n)	Total (n)
HLA-A*0101 / VTEHDTLLY / PE	16(5)	26(25)	42(30)
HLA-A*0201 / NLVPMVATV / PE	41(15)	37(34)	78(49)
HLA-B*0701 / TPRVTGGGAM / PE	13(2)	14(14)	27(16)
HLA-B*0801 / ELRRKMMYM / PE	7(2)	10(8)	17(10)
HLA-B3501 / IPSINVHHY / PE	14(5)	10(7)	24(12)
Total	91(29)	97(88)	188(117)

The method comparison was evaluated using only samples with CMV-specific T cells results >1 cell/ $\mu$ L.

Dextramer CMV Kit vs. Predicate iTAg Tetramer

CMV-specific T cells enumeration results with samples of >1 cell/ $\mu$ L

T subset	Slope	95% CI	Intercept	95% CI	r
CD3+CD8+	0.9840	0.92 to 1.05	-0.4406	-8.47 to 7.59	0.905
CMV-specific CD8+T-cell	1.010	0.89 to 1.13	0.39	0.09 to 0.69	0.952

Agreement with predicate: All Samples

Method		Predicate iTAg Tetramer		
		$\geq 7$ cells/ $\mu$ L	<7 cells/ $\mu$ L	Total
Test Dextramer CMV Kit	$\geq 7$ cells/ $\mu$ L	33	8	41
	<7 cells/ $\mu$ L	5	142	147
	Total	38	150	188

Positive Percent Agreement (33/38): 86.8% (95% CI: 71.9–95.6%)

Negative Percent Agreement (142/150): 94.7% (95% CI: 89.8–97.7%)

Agreement with predicate: Samples with CMV-specific T cells of  $\geq 1$  cell/ $\mu$ L

Method		Predicate iTAg Tetramer		
		$\geq 7$ cells/ $\mu$ L	<7 cells/ $\mu$ L	Total
Test Dextramer CMV Kit	$\geq 7$ cells/ $\mu$ L	33	8	41
	<7 cells/ $\mu$ L	5	71	76
	Total	38	79	117

Positive Percent Agreement (33/38): 86.8% (95% CI: 71.9–95.6%)

Negative Percent Agreement (71/79): 89.9% (95% CI: 81.0–95.5%)

Sample range for CD3+CD8+ is 2.90–1389 CD3+CD8+ T cells/ $\mu$ L.

Sample range for CMV-specific CD8+ T-cells is 1.01–93.87 CMV-specific CD8+ T cells/ $\mu$ L.

*b. Matrix comparison:*

Only Sodium (Na) heparin was used in this new device as well as with the predicate device.

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):*

Risk Assessment:

A prospective study was performed with 120 patients with an allogeneic stem cell transplant (SCT) followed for up to one year for recurrence of CMV infection and determination of numbers of CMV-specific CD8+ T cells using the CMV Dextramer Kit. Dextramer analysis was performed pretransplant, at days 30, 100, and 365 and included the alleles HLA-A\*0101, A\*0201, B\*0702, B\*0801, and B\*3501. The Dextramer giving the highest absolute counts at each timepoint for each patient was used in analysis and evaluation.

In accordance with routine practice Antigenemia tests were performed weekly from Day 21 and continuing at least until Day 120 or until the patient was off all immunosuppression.

Relative risk was evaluated based on results at Day 100 from patients that were both CMV seropositive and reactivated CMV at least one time. Day 100 was determined to be the best time point to assess recovery. At day 30, 90% of the patients did not have any measurable CMV-specific T-cells and none of the patients had reactivated CMV. There were insufficient numbers of patients in the study at day 365 to make a conclusion about the performance of the device at that time point.

Fewer patients had results at day 365 as compared to day 100 and the patients were not followed after the last time point.. Five patients that reactivated CMV had no CMV Dextramer results obtained at day 100 and were excluded from the study. Fourteen of the remaining 29 patients had one or more CMV reactivations post day 100 (recurrent viremia group) while 15 patients did not develop CMV infection after day 100 (resolved group). The average number of CMV-specific T cells in the resolved group was significantly higher (median=47 cells/ $\mu$ L) compared to the group with recurrent viremia (median=13 cells/ $\mu$ L).

In summary, 19 patients had recovered CMV-specific CD8+ T cell immunity (levels  $\geq 7$  cells/ $\mu$ L) at day 100 and 74% (14/19) of these patients did not develop CMV infection after day 100. Of the remaining patients, that had  $< 7$  cells/ $\mu$ L at day 100, 90% (9/10) developed CMV infection after day 100.

CMV seropositive patients that had one or more CMV reactivations

		Recurrent CMV Infection post Day100		
		No	Yes	Total
CMV Specific CD8+ T cells	Delayed Recovery $< 7$ cells/ $\mu$ L	1	9	10
	Rapid Recovery $\geq 7$ cells/ $\mu$ L	14	5	19
	Total	15	14	29

All samples

		Recurrent Viremia		
		No	Yes	Total
CMV Specific CD8+ T cells	Delayed Recovery $< 7$ cells/ $\mu$ L	77	14	91
	Rapid Recovery $\geq 7$ cells/ $\mu$ L	9	20	29
	Total	86	34**	120***

\* Eight samples were excluded from the final analysis due to HLA alleles did not match the claimed alleles.

\*\* 34 patients developed antigenemia, however five patients were excluded because they did not have a sample taken at Day 100.

\*\*\* 52 patients were excluded for being CMV seronegative and had CMV seronegative donors

Risk of developing CMV antigenemia after Day 100

Risk is defined as the risk of a patient with less than 7 cells/ $\mu$ L developing CMV infection post Day 100 compared to a patient with more than 7 cells/ $\mu$ L.

# CMV+ T cells at Day 100	Development of antigenemia (post Day 100)		Total
	Yes	No	
<7 cells/ $\mu$ L	9	1	10
$\geq$ 7 cells/ $\mu$ L	5	14	19
Total	14	15	29
Risk	3.4 (95% CI: 1.57 – 7.46)		

Dextramer results were evaluated at Day 30, 100, and Day 365 post-transplant. Only samples collected on day 100 showed significant association between CMV-specific CD8+ T cells status and development of antigenemia. Results summarized in the table above show that the relative risk in developing antigenemia is 3.4 (95% CI: 1.57 – 7.46) for patients with < 7 cells/ $\mu$ L CMV-specific CD8+ T cells determined by the Dextramer CMV kit, as compared to patients with  $\geq$  7 cells/ $\mu$ L CMV-specific CD8+ T cells.

4. Clinical cut-off:

CMV dextramer+CD8+ T cells >7 cells/ $\mu$ L at Day 100 post-transplant

5. Expected values/Reference range:

The study to determine the expected reference range of CMV-specific T-cells results obtained with CMV Dextramers was performed using 55 CMV-seropositive and 62 CMV-seronegative stem cell transplant patients. 98% (61/62) of CMV-seronegative patients have undetectable (< 1 cell/  $\mu$ L) CMV-specific T cells ranging from 0.00 – 0.25 cells/ $\mu$ L and 56% (31/55) of CMV seropositive patients have detectable (> 1 cell/  $\mu$ L) CMV-specific T-cells ranging from 1.15 – 182 cells/  $\mu$ L. Samples tested may test negative for CMV-specific T-cells as the CMV response may be restricted to non-claimed alleles that are not in the scope of this clearance The CMV Dextramer Kit is intended for use with five claimed alleles.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.