IMMUNOLOGY TAKEN TO A HIGHER LEVEL

CE

Dextramer® CMV Kit	**Cat. No. CX01**
HLA-A*0101 / VTEHDTLLY / PE	Cat. No. CA2131-PE
HLA-A*0201 / NLVPMVATV / PE	Cat. No. CB2132-PE
HLA-A*0301 / KLGGALQAK / PE	Cat. No. CC2197-PE
HLA-A*2402 / QYDPVAALF / PE	Cat. No. CF2133-PE
HLA-B*0702 / RPHERNGFTVL / PE	Cat. No. CH2135-PE
HLA-B*0702 / TPRVTGGGAM / PE	Cat. No. CH2136-PE
HLA-B*0801 / ELRRKMMYM / PE	Cat. No. CI2137-PE
HLA-B*3501 / IPSINVHHY / PE	Cat. No. CK2138-PE
Negative control / PE	Cat. No. CI3233-PE

Intended Use

Dextramer® CMV Kit is intended for the identification and enumeration of cytomegalovirus (CMV)-specific $CD8^+$ T cells in whole blood by flow cytometry.

Dextramer® CMV Kit is indicated for use for the assessment of CMV-specific immune status.

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, A*0301, A*2402, B*0702, B*0801, B*3501.

Summary and Explanation

Cytomegalovirus (CMV) is a herpes virus that infects 50-85% of the adult population and remains latent in healthy individuals through control by the presence of CMV-specific T cells. CMV-specific $CD8^+$ T cells play a critical role in suppressing CMV reactivation. In healthy individuals an equilibrium is achieved where CMV-specific T cells control the persisting virus. When T cell function is impaired and equilibrium is not established, viral reactivation and clinical disease may develop.

Reactivation of CMV is a frequently occurring complication of immunosuppresion in transplant patients and can significantly contribute to morbidity and mortality if the virus is not controlled.

CMV reactivation may be controlled by preemptive antiviral therapy, but this is often hampered by side-effects, renal toxicity and drug-resistant strains.

Several studies have demonstrated an increased risk of CMV viremia or disease in patients with low levels of specific T-cell immunity, and likewise, the development of T cell immunity has been shown to be associated with decreased risk of CMV viremia and disease after transplantation[1-4] Enumeration of CMV-specific $CD8^+$ T cells provide information about the status of the CMV-specific immune response and can predict patients in high risk of developing CMV disease[1-3]

Detection of CMV-specific $CD8^+$ T cells requires recognition of the T-cell receptor (TCR) by a unique combination of a MHC class I molecule coupled with a CMV-specific peptide. CMV-specific TCR's on the surface of $CD8^+$ T cells are recognized by CMV Dextramers. CMV Dextramers comprise dextran polymer backbone carrying multiple MHC-peptide complexes and fluorochrome molecules (PE). Together with fluorescent-labeled anti-CD3 and anti-CD8 antibodies CMV Dextramers are used for detection and enumeration of CMV-specific $CD3^+CD8^+$T cells by flow cytometry.

T cell immune response to CMV varies between individuals, is dependent on HLA-type, and is influenced by HLA composition. CMV-specific cellular immune responses restricted by some alleles dominate those restricted by others[6]. It is therefore important to measure CMV-specific immune responses restricted by as many HLA alleles as possible in a given individual.

The Dextramer® CMV Kit comprises 8 different CMV Dextramers representing 7 different alleles covering ~95% of the European population.

Principle of Procedure

The CMV Dextramers accurately detect and quantify CMV-specific T cells in blood samples. This involves a two-step procedure:

- Step 1: Determination of the percentage of CMV-specific $CD3^+CD8^+$ T cells in the sample (Tube A)
- Step 2: Determination of the absolute number of $CD3^+CD8^+$ T-cells in the sample (Tube C)

The absolute number of CMV-specific $CD3^+CD8^+$ T cells can then be determined (see Interpretation of Results below).

Reagents

Materials provided
The Dextramer® CMV Kit comprises the following reagents:

HLA-A*0101 / VTEHDTLLY / PE	25 tests/0.25 ml	Cat. No. CA2131-PE
HLA-A*0201 / NLVPMVATV / PE	50 tests/0.50 ml	Cat. No. CB2132-PE
HLA-A*0301 / KLGGALQAK / PE	25 tests/0.25 ml	Cat. No. CC2197-PE
HLA-A*2402 / QYDPVAALF / PE	25 tests/0.25 ml	Cat. No. CF2133-PE
HLA-B*0702 / RPHERNGFTVL / PE	25 tests/0.25 ml	Cat. No. CH2135-PE
HLA-B*0702 / TPRVTGGGAM / PE	25 tests/0.25 ml	Cat. No. CH2136-PE
HLA-B*0801 / ELRRKMMYM / PE	25 tests/0.25 ml	Cat. No. CI2137-PE
HLA-B*3501 / IPSINVHHY / PE	25 tests/0.25 ml	Cat. No. CK2138-PE
Negative control / PE	150 tests/1.50 ml	Cat. No. CI3233-PE

The reagents are also available as individual reagents with 50 tests/0.50 ml per vial.
Materials required but not provided

Flow tube, 12 x 75 mm, disposable tubes recommended for flow cytometry

Anti-CD8/FITC, clone SK1 (BD Cat. No. 345772)
Anti-CD4/PE, clone SK3 (BD Cat. No. 345769)
Anti-CD3/PerCP, clone SK7 (BD Cat. No. 345766)
Truecount tubes (BD Cat. No. 340334)
FACS Lysing Solution (10X) (BD, Cat. No. 349202)
PBS (e.g. 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4; pH = 7.4)
Fixing solution (e.g. 2% Methanol free formalin in PBS)

Centrifuge capable of 400 x g
Pipette

Flow cytometer with excitation light source and 3 independent fluorescence channels for FITC, PE and PerCP, and capable of forward scatter (FS) and side scatter (SS) detection.

Storage and Preparation of Kit Components

Always keep CMV Dextramers stored at 2-8°C in the dark– the brown plastic vial does not protect the reagent sufficiently against light.

Precautions

For *in vitro* diagnostic use.

For professional users.

Specimens, before and after preparation, and all materials exposed to them, should be handled as if capable of transmitting infection and should be disposed of with proper precautions[7] .

CMV Dextramers contain sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

All materials should be disposed of according to your institution's guidelines for hospital waste disposal.

Specimen Collection and Preparation

Collect blood by venipuncture into a blood collection tube containing an appropriate anti-coagulant (EDTA, Citrate or Heparin). Collected blood should be analyzed within 24 hours.

FACS Lysing Solution:
Dilute the FACS Lysing Solution (10X concentrate) 1:10 with room temperature (20° to 25°C), deionized water. Store prepared solution as recommended by the manufacture.

Test Procedure

Assay Procedure
Select a CMV Dextramer matching the HLA-type of the patient. If multiple CMV Dextramers are applicable, select all and make analysis for each allele.

Tube A + B (Assessment of % CMV-specific T cells):

1. Pipette 200 µl anti-coagulated whole blood in a 12 x 75 mm flow tube.
2. Add 10 µl appropriate CMV Dextramer to Tube A and 10 µl Negative control / PE to Tube B and incubate for 10 min. at room temperature in the dark. If a blood sample is analyzed by more than one CMV Dextramer, prepare separate Tube A for each CMV Dextramer.
3. Add 10 µl anti-CD8/FITC and 10 µl anti-CD3/PerCP to both Tube A and Tube B.
4. Incubate for 30 min. on ice in the dark.
5. Add 2 mL of 1x FACS Lysing Solution. Vortex gently and incubate for 10 min. in the dark at room temperature.
6. Centrifuge 400 x g for 5 min., pour off supernatant and resuspend cell pellet in 2 ml PBS.
7. Centrifuge 400 x g for 5 min., pour off supernatant and resuspend cell pellet in 300-400 µl Fixing solution.
8. Store samples at 2-8°C in the dark until analysis on flow cytometer (samples can be run up to 24 hours after lysis).
9. Acquire 25.000 dual $CD3^+$ and $CD8^+$ events.

Tube C (Assessment of absolute count of $CD3^+CD8^+$ cells):

1. Add 100 µl anti-coagulated whole blood in a TruCOUNT Tube.
2. Add 10 µl anti-CD8/FITC, 10 µl anti-CD4/PE and 10 µl anti-CD3/PerCP.
3. Incubate for 30 min. at 2-8°C in the dark.
4. Add 1 mL of 1x FACS Lysing Solution. Vortex gently and incubate for 10 min. in the dark at room temperature.
5. Store samples at 2-8°C in the dark until analysis on flow cytometer (samples can be analyzed up to 6 hours after lysis).
6. Acquire 10.000 bead events, using a threshold set on $CD3^+$ cells.

Quality Control

Flow cytometer: Follow manufacturer recommendations for daily flow cytometer instrument set-up and daily instrument quality assurance for three-color immunophenotyping[5].
Method: Use commercial whole blood controls providing established values for percent positive $CD4^+$ and $CD8^+$ cells with each run to assess system performance. The control cells should be stained as described for Tube C. The values of the two subsets must fall within the expected range stated by the provider.

Control between tubes: $CD8^+$ results expressed as a percentage of $CD3^+$ should be $\leq$ 5% between Tube A and Tube C.

Background: Tube B is used to evaluate background. The percentage of Dextramer-specific T cells should be <0.2% of $CD8^+$ T cells.

Procedural Notes
The addition of a precise volume of blood is critical to achieve reliable results. Use electronical pipettes that operate in the reverse pipetting mode or perform the reverse pipetting technique using manual pipettes.

Acquisition protocols

Before acquiring samples adjust the threshold to include cell and bead populations of interest and minimize debris. Use same instrument settings for Tube A, B and C.

Make protocols that allow the following dot plot figures to be made:

Tube A + B (Assessment of % CMV-specific T cells):

A) FS vs. SS: Ensure lymphocyte population is visible. Draw exclusion gate on low scatter debris (region R1)
B) Anti-CD3 vs. SS: exclude region R1, draw gate around $CD3^+$ cells (region R2)
C) FS vs. SS: exclude region R1, include region R2, draw gate on lymphocytes (region R3)
D) Anti-CD3 vs. anti-CD8: exclude region R1, include region R2 + R3, draw gate on $CD8^+$ cells (region R4)
E) CMV Dextramer vs. anti-CD8: exclude region R1, include region R2 + R3 + R4, draw gate around CMV^+ cells (region R5)

Acquire 25.000 $CD3^+CD8^+$ events in region R4.

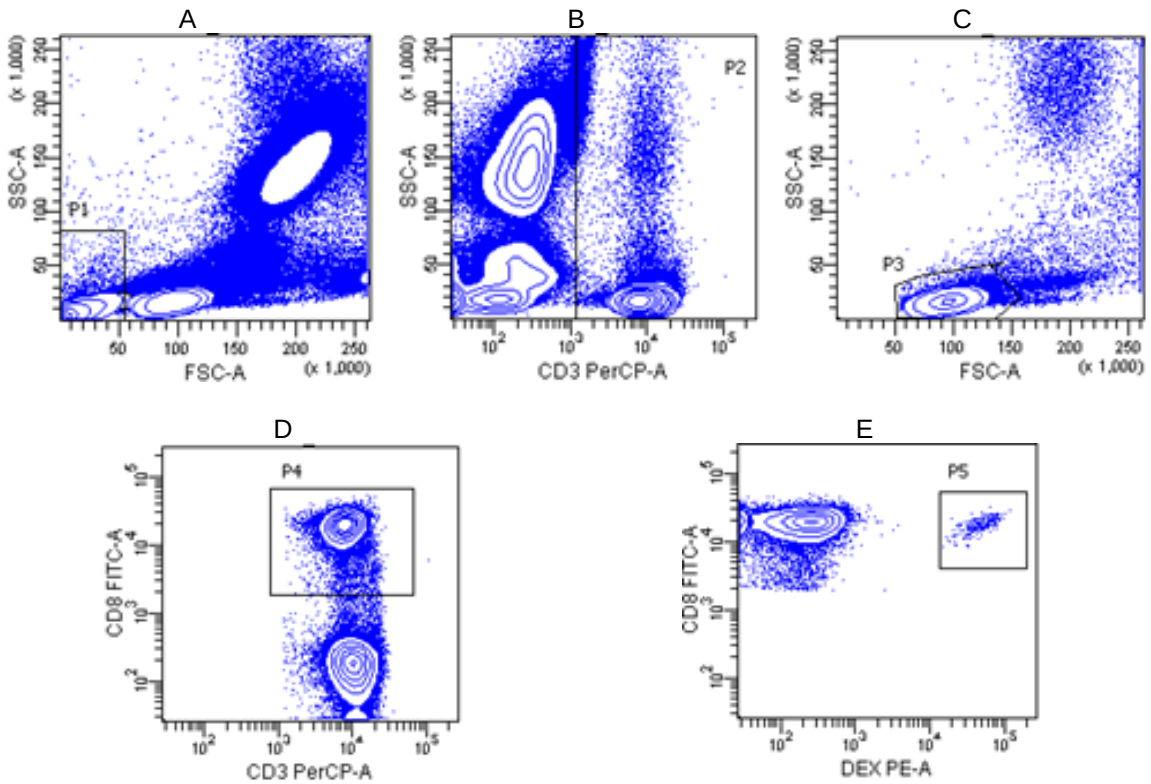


Figure 1. Dot plots tube A and B. Illustrative example from FACSCanto II using Diva software

Tube C (Assessment of absolute count of $CD3^+CD8^+$ cells):

F) Anti-CD3 vs. SS: Set a threshold excluding CD3 negative events. Ensure whole population of both beads and lymphocytes are visible in included area. Draw gate on $CD3^+$ cells (region R6)
G) FS vs. SS: include region R6, draw gate on lymphocytes (region R7)
H) Anti-CD3 vs. anti-CD8: include region R6 + R7, draw gate on $CD8^+$ cells (region R8)
I) Anti-CD4 vs. anti-CD8: Ungated, draw gate on bead events (region R9).

Acquire 10.000 bead events in region R9.

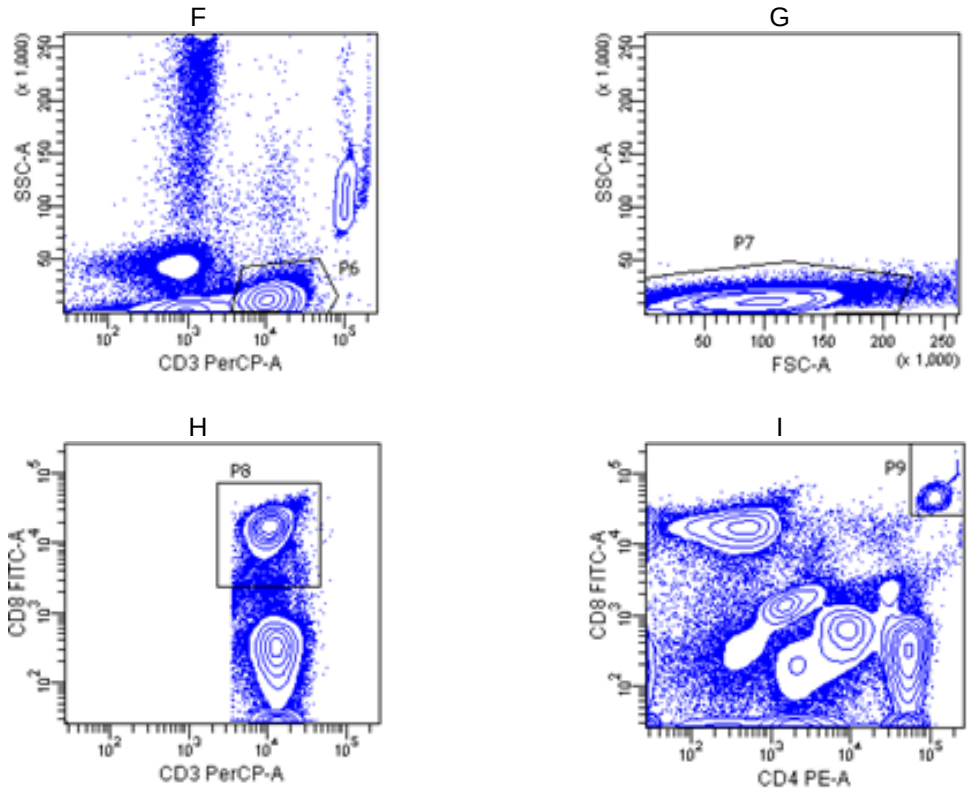


Figure 2. Dot plots tube C. Illustrative example from FACSCanto II using Diva software

Interpretation of Results
Determine the following values:

Value	Tube	Region	Purpose
# $CD3^+CD8^+$	C	R8, plot H	Calculation of absolute count of $CD3^+CD8^+$ cells/ µl blood
# Bead events	C	R9, plot I	Calculation of absolute count of $CD3^+CD8^+$ cells/ µl blood
%$CD3^+CD8^+$	C	R8, plot H	Reproducibility check between Tube C and Tube A
%$CD3^+CD8^+$	A	R4, plot D	Reproducibility check between Tube C and Tube A
%$CD3^+CD8^+CMV^+$	A	R5, plot E	Calculation of absolute count of $CD3^+CD8^+CMV^+$ cells/ µl
%$CD3^+CD8^+CMV^+$	B	R5, plot E	Determination of background staining

1) Calculate absolute counts of $CD3^+CD8^+$ T cells in Tube C. Use the equation:

$$\text{Absolute count } CD3^+CD8^+ \text{ cells} = \frac{CD3^+CD8^+\text{events (region R8)}}{\text{bead events (region R9)}} \text{ x } \frac{\text{bead events per test}}{\text{whole blood volumen tested (µl)}}$$

2) Determine percentage of CMV-specific $CD3^+CD8^+$ T cells in Tube A:

% CMV Dextramer positive events in region R5

3) Calculate absolute number of CMV-specific T cells in blood using the equation:

$$\text{Absolute count } CMV^+ \text{ T cells} = \frac{(\text{absolute count } CD3^+CD8^+\text{cells (step 1)}) \text{x } (\%\ \text{CMV Dex}^+\text{T cells (step 2)})}{100}$$

Limitations

The use of the Dextramer® CMV Kit is limited to individuals with at least one of the following HLA types: A*0101, A*0201, A*0301, A*2402, B*0702, B*0801, B*3501. It cannot be used for individuals with HLA-type different from those.

The Dextramer® CMV Kit does not measure CMV infection or disease.

Individual CMV Dextramers can only be used on samples with matching HLA-types.

Performance Characteristics

Detection limit – Analytical Sensitivity
5 routine blood specimens from 5 stem cell transplant recipients representing 4 alleles were included in the study. Serial dilutions of each specimen were tested in triplicates using the HLA-matched CMV Dextramer.

	HLA-B*0702		HLA-A*0201		HLA-A*0301		HLA-B*0801		HLA-A*0201	
Dilution	Mean (cells/µl)	CV (%)	Mean (cells/µl)	CV (%)	Mean (cells/µl)	CV (%)	Mean (cells/µl)	CV (%)	Mean (cells/µl)	CV (%)
100%	83,78	2	229,93	0	127,97	1	655,34	2	31,36	1
56,23%	46,95	4	107,32	2	69,42	2	559,13	1	9,11	6
31,62%	27,85	6	53,29	2	38,06	3	464,85	2	3,17	3
17,78%	15,91	2	29,53	2	21,70	3	341,62	2	1,61	12
10%	9,43	4	16,16	2	11,13	13	234,83	0	0,85	11
5,60%	5,38	6	9,64	5	7,19	9	154,89	2	0,50	26
3,20%	3,12	4	4,97	6	3,99	1	91,78	7	0,24	39
1,80%	1,98	15	2,99	7	2,03	17	52,72	4	0,18	24
1%	1,05	18	1,61	0	1,44	9	28,67	6	0,11	31
0,56%	0,53	13	0,94	6	0,59	23	16,45	12	0,07	25
0,32%	0,45	42	0,74	16	0,38	35	11,16	19	0,08	22
0,18%	0,20	35	0,81	22	0,21	49	6,83	11	0,01	173
0,10%	0,16	43	0,27	22	0,21	49	4,04	33	0,00	-
0,06%	0,20	35	0,24	25	0,15	35	2,41	14	0,02	87
0,03%	0,12	0	0,10	100	0,09	0	2,41	14	0,00	-
0	0,00	-	0,07	87	0,00	-	0,77	57	0,00	-

The limit of detection (LoD) is 1 cells/µl as determined by the lowest concentration of cells (cells/µl) that can be determined with a CV% below 20%.

Analytical specificity

16 CMV-seronegative routine blood specimens from 9 stem cell transplant recipients representing 7 alleles were included in the study. Each specimen was tested using the HLA-matched CMV Dextramer.

Specimen (HLA type)	**MHC Dextramer**	**Result** (cells/µl)
A*0101	HLA-A*0101 / VTEHDTLLY / PE	0.00
A*0101	HLA-A*0101 / VTEHDTLLY / PE	0.00
A*0201	HLA-A*0201 / NLVPMVATV / PE	0.00
A*0201	HLA-A*0201 / NLVPMVATV / PE	0.03
A*0301	HLA-A*0301 / KLGGALQAK / PE	0.00
A*0301	HLA-A*0301 / KLGGALQAK / PE	0.00
A*2402	HLA-A*2402 / QYDPVAALF / PE	0.00
A*2402	HLA-A*2402 / QYDPVAALF / PE	0.00
B*0702	HLA-B*0702 / RPHERNGFTVL / PE	0.00
B*0702	HLA-B*0702 / RPHERNGFTVL / PE	0.00
B*0702	HLA-B*0702 / TPRVTGGGAM / PE	0.00
B*0702	HLA-B*0702 / TPRVTGGGAM / PE	0.00
B*0801	HLA-B*0801 / ELRRKMMYM / PE	0.00
B*0801	HLA-B*0801 / ELRRKMMYM / PE	0.00
B*3501	HLA-B*3501 / IPSINVHHY / PE	0.00
B*3501	HLA-B*3501 / IPSINVHHY / PE	0.00
	Mean	0.00
	CV%	0.01
	Max.	0.03
	Min.	0.00

The analytical specificity is 100% (16/16) as all negative results are within 0.00 – 0.03 cells/µl and thus below the limit of detection of 1 cell/µl.

Linearity - Assay reportable range

6 routine blood specimens from 6 stem cell transplant recipients representing 4 alleles were included in the study. Serial dilution of each specimen was tested in triplicate using the HLA-matched CMV Dextramer.

		A*0201		**B*0801**		**A*0201**	
Dilution	N	Cells/µl expected	Cells/µl actual	Cells/µl expected	Cells/µl actual	Cells/µl expected	Cells/µl actual
100,00%	3	**31,36**	**31,36**	**66,77**	**66,77**	**229,93**	**229,93**
56,23%	3	**17,63**	**9,11**	**37,54**	**29,46**	**129,29**	**107,32**
31,62%	3	**9,92**	**3,17**	**21,11**	**14,98**	**72,70**	**53,29**
17,78%	3	**5,58**	**1,61**	**11,87**	**7,74**	**40,88**	**29,53**
10,00%	3	3,14	0,85	**6,68**	**4,21**	**22,99**	**16,16**
5,60%	3	1,76	0,50	**3,74**	**2,26**	**12,88**	**9,64**
3,20%	3	1,00	0,31	**2,14**	**1,49**	**7,36**	**4,97**
1,80%	3	0,56	0,18	1,20	0,72	**4,14**	**2,99**
1,00%	3	0,31	0,11	0,67	0,55	**2,30**	**1,61**
0,56%	3	0,18	0,07	0,37	0,28	1,29	0,94
0,32%	3	0,10	0,08	0,21	0,06	0,74	0,74
0,18%	3	0,06	0,01	0,12	0,03	0,41	0,81
0,10%	3	0,03	0,00	0,07	0,03	0,23	0,27
0,06%	3	0,02	0,03	0,04	0,03	0,14	0,24
		R^2 = 0.950		R^2 = 0.984		R^2 = 0.988	
		Slope = 1.18		Slope = 0.996		Slope = 0.978	
		A*0301		**B*0801**		**B*0702**	
Dilution	N	Cells/µl expected	Cells/µl actual	Cells/µl expected	Cells/µl actual	Cells/µl expected	Cells/µl actual

100,00%	3	**127,97**	**127,97**	-	655,34	**83,78**	**83,78**
56,23%	3	**71,96**	**69,42**	-	559,13	**47,11**	**46,95**
31,62%	3	**40,46**	**38,06**	-	464,85	**26,49**	**27,85**
17,78%	3	**22,75**	**21,70**	-	341,62	**14,90**	**15,91**
10,00%	3	**12,80**	**11,13**	**234,83**	**234,83**	**8,38**	**9,43**
5,60%	3	**7,17**	**7,19**	**132,04**	**154,89**	**4,69**	**5,38**
3,20%	3	**4,09**	**3,99**	**74,25**	**91,78**	**2,68**	**3,12**
1,80%	3	**2,30**	**2,03**	**41,75**	**52,72**	**1,51**	**1,98**
1,00%	3	**1,28**	**1,44**	**23,48**	**28,67**	**0,84**	**1,05**
0,56%	3	0,72	0,59	**13,15**	**16,45**	0,47	0,53
0,32%	3	0,41	0,38	**7,51**	**11,16**	0,27	0,45
0,18%	3	0,23	0,21	**4,23**	**6,83**	0,15	0,20
0,10%	3	0,13	0,21	**2,35**	**4,04**	0,08	0,16
0,06%	3	0,08	0,15	**1,32**	**2,41**	0,05	0,20
		R^2 = 0.999 Slope = 0.995		R^2 = 0.991 Slope = 1.03		R^2 = 0.999 Slope = 0.99	

The results show that the Dextramer® CMV Kit has a linear reportable range of 1 – 250 cells/µl.

Interference – Mismatch-Cross-Reactivity

Studies have shown no interference from monocytes, granulocytes, or platelets. Also, no cross-reaction was observed in studies using HLA mis-matched CMV Dextramer.

Precision – Reproducibility - Assay portability

Intra-'Dextramer' reproducibility

16 routine blood specimens from 16 stem cell transplant recipients representing 6 alleles were included in the study. Each specimen was tested 10 times using Tube A with HLA-matched CMV Dextramer and 1 time using Tube C to study the reproducibility of the CMV Dextramer part.

HLA Type	CMV Dextramer	Mean (cells/µl)	STDEV (cells/µl)	CV (%)
A*2402	HLA-A*2402 / QYDPVAALF / PE	1.11	0.17	15
A*0101	HLA-A*0101 / VTEHDTLLY / PE	26.07	1.93	7
B*0702	HLA-B*0702 / RPHERNGFTVL / PE	2.25	0.25	11
B*0702	HLA-B*0702 / TPRVTGGGAM / PE	4.01	0.30	8
A*0201	HLA-A*0201 / NLVPMVATV / PE	13.20	0.66	5
A*0201	HLA-A*0201 / NLVPMVATV / PE	3.13	0.25	8
B*0702	HLA-B*0702 / RPHERNGFTVL / PE	5.32	0.18	3
B*0702	HLA-B*0702 / TPRVTGGGAM / PE	10.91	0.32	3
A*0101	HLA-A*0101 / VTEHDTLLY / PE	10.96	0.56	5
A*0101	HLA-A*0101 / VTEHDTLLY / PE	1.75	0.14	8
A*0301	HLA-A*0301 / KLGGALQAK / PE	2.77	0.30	11
B*0801	HLA-B*0801 / ELRRKMMYM / PE	0.94	0.11	11
A*0201	HLA-A*0201 / NLVPMVATV / PE	21.97	0.47	2
A*0301	HLA-A*0301 / KLGGALQAK / PE	3.68	0.25	7
B*0801	HLA-B*0801 / ELRRKMMYM / PE	10.53	0.45	4
B*0801	HLA-B*0801 / ELRRKMMYM / PE	513.13	4.77	1

Intra-lab reproducibility

A panel of 3 samples prepared from specimens from 2 healthy human donors representing 3 alleles with medium, low, and negative CMV T-cell response were included in the study. Each sample was tested 10 times by two separate operators (1 and 2).

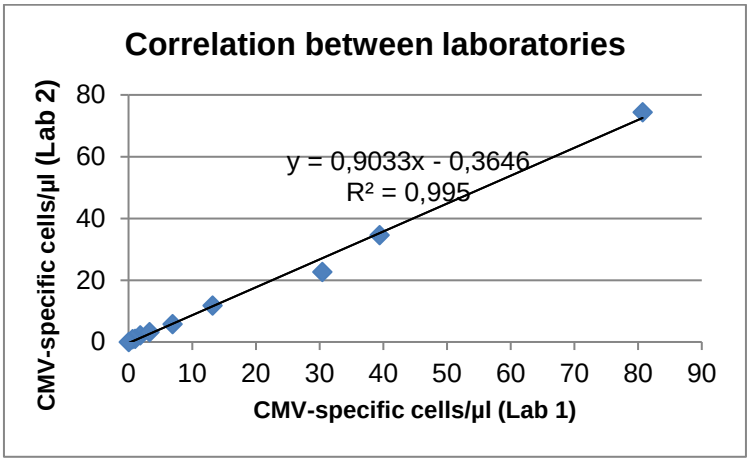
HLA Type	CMV Dextramer	Operator	CMV T cell	Mean (cells/µl)	STDEV (cells/µl)	CV (%)
A*0101	HLA-A*0101 / VTEHDTLLY / PE	1	Medium	25.72	2.01	8
		2		24.48	1.26	5
A*0201	HLA-A*0201 / NLVPMVATV / PE	1	Low	1.45	0.18	12
		2		1.50	0.17	11
B*0801	HLA-B*0801 / ELRRKMMYM / PE	1	Negative	0.54	-	-
		2		0.39	-	-

Inter-lab reproducibility

A panel of 3 samples prepared from specimens from healthy human donors representing 3 alleles was mixed with negative donor whole blood to simulate samples with high, medium, low and negative CMV T-cell response. A total of 9 samples were included in the study. Each sample was tested once with HLA-matched CMV Dextramer at 2 individual sites.

HLA Type	CMV Dextramer	CMV T cell	Lab 1 (cells/µl)	Lab 2 (cells/µl)
A*0201	HLA-A*0201 / NLVPMVATV / PE	Medium	1,8	2,1
		Low	1,0	1,0
		Negative	0,6	0,9
A*0101	HLA-A*0101 / VTEHDTLLY/ PE	High	39,4	34,6
		Medium	13,2	11,8
		Low	3,3	3,2
B*0801	HLA-B*0801 / ELRRKMMYM / PE	High	80,7	74,4
		Medium	30,4	22,7
		Low	6,9	5,8

Linear regression analysis as shown below indicates that results obtained at the 2 sites are substantial equivalent.

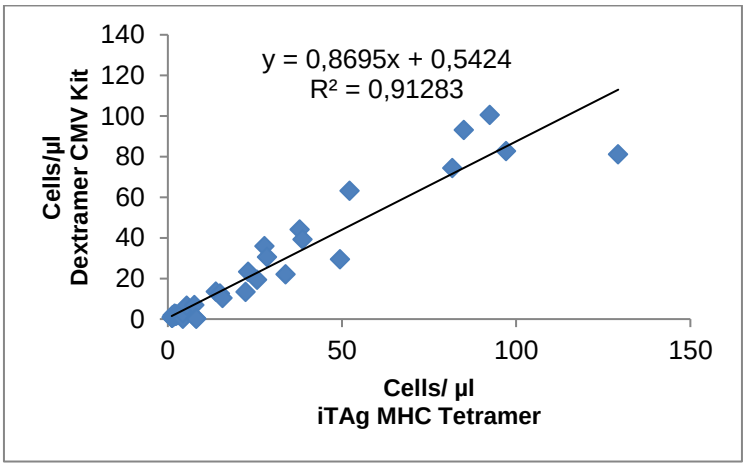


Enumeration of CMV-specific T cells - Assessment of Immune status

The association between delayed CMV-specific T-cell response and increased risk of CMV reactivation in transplantation patients is well supported by international consensus guidelines[4] and clinical studies[1-3]. In these studies, the clinical cut-off for a protective CMV-specific T-cell response is in the range of 2-10 cells/µL.

158 routine blood specimens from 75 stem cell transplant recipients representing 6 alleles were included in the below study. Each specimen was tested using CMV-specific iTAg MHC Tetramer and corresponding CMV Dextramer in accordance with the manufacturer's instructions.

The number of CMV-specific T cells for samples with CMV-specific T-cell response above 1 cell/µl was determined for both iTAg MHC Tetramer and CMV Dextramer and results compared by linear regression analysis.



The study were also used to compare iTAg MHC Tetramer results and Dextramer® CMV Kit results for the assessment of CMV-specific immune status and risk of CMV reactivation using 2 -10 cells/µL as clinical cut-off, such that results in the range of 2 -10 cells/µL are inconclusive.

		Dextramer CMV Kit		
		> 10 cells/µL	2-10 cells/µL	< 2 cells/µL
iTAg MHC Tetramer CMV Kit	> 10 cells/µL	18	0	0
	2-10 cells/µL	0	6	5
	< 2 cells/µL	0	2	129

Positive Agreement: 100% (18/18), 95% CI: 84.7-100%

Negative Agreement: 100% (129/129), 95% CI: 97.7-100%

Inconclusive (iTAg MHC Tetramer): 7% (11/158)

Inconclusive (Dextramer® CMV Kit): 5% (8/158)

Troubleshooting

If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our customer services

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