

CMV-Tetramer TEST

to better identify patients at risk for recurrent CMV infection and CMV disease:

Monitoring of cellular immune response with CMV-specific Tetramers in whole blood.




Monitoring CMV-specific CD8+ T cell numbers – a 2 step procedure

Step 1: Assessment of absolute CD8+ T cell counts using counting beads

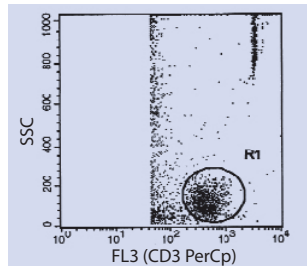
1. Addition of antibodies

add CD3-PerCp, CD8-FITC, CD4-PE
5 µl each



Counting Tube

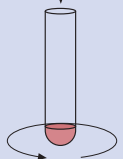
The lypophilisate of the counting beads is dissolved by addition of the antibodies



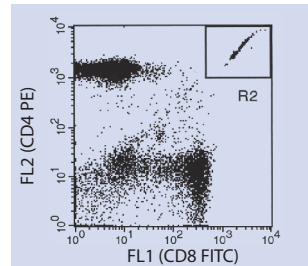
FL3 (CD3-PerCp) versus SSC dot plot. Set region on CD3+ T-Lymphocytes (R1). Set threshold on FL3 to minimize debris

2. Addition of whole blood

+ 100 µl EDTA whole blood, vortex gently to mix
incubate 20 min at RT



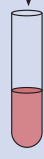
Accuracy is critical
Perform reverse pipetting: This technique takes advantage of the two stops of a pipette
Reverse pipetting: Depress the button to the second stop. When the button is released, excess sample is drawn up into the tip. Precise volume of sample is expelled by pressing the button to the first stop, leaving excess sample in the tip.



Ungated CD4 versus CD8 dot plot. Set gate on counting beads (R2)

3. Lyse – no wash

+ 2 ml LYSING SOLUTION



Vortex and incubate for 15 min in the dark at RT
Do not wash
Perform Analysis

Data Evaluation

(1) Calculating absolute counts of CD8 T-lymphocytes: Determine the number of CD8+ T-lymphocytes in region 3 and the number of counting beads in region 2.

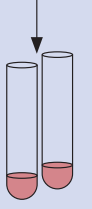
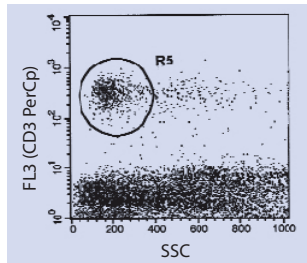
If you are not using a BD software program that automatically calculates absolute cell counts, you can perform a manual calculation using the following equation: $\frac{\# \text{ of CD3/CD8 events in R3}}{\# \text{ of beads in R2}} \times \frac{\# \text{ of beads per test}^*}{\text{test volume (100 } \mu\text{l)}} = \text{absolute count of CD3/CD8 cells}/\mu\text{l}$

* This value is found on the TruCOUNT Absolute Count Tube foil pouch label

Step 2: Assessment of % Tetramer binding T-lymphocytes

1. Addition of whole blood

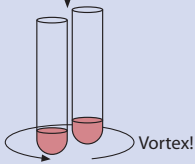
Patient: 2 x 200 µl whole blood

Ungated SSC versus CD3-PerCP (FL3). Set region on CD3+ lymphocytes (R5)

2. Addition of Tetramer

add 10 µl CMV-tetramer to tube 1 and 10 µl negative Tetramer to tube 2 – vortex and incubate 30 min at RT



Vortex!

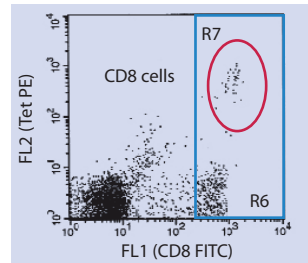
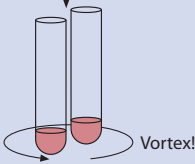


Fig 1: R5 gated CD8-FITC versus Tetramer-PE. Set region on all CD8 cells (R6) and on Tetramer counts (R7)

3. Addition of CD3 and CD8

add 5 µl CD3-PerCp + 5 µl CD8-FITC – vortex – stain 20 min at RT in the dark



Vortex!

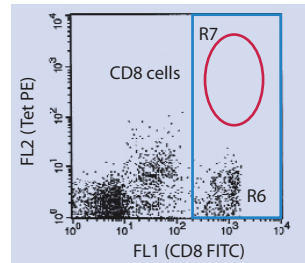
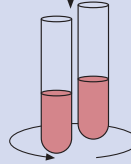


Fig 2: R5 gated CD8-FITC versus negative Tetramer-PE. Copy R6 and R7 and paste into dot plot.

4. Lyse – wash

+ 2 ml LYSING SOLUTION incubate 20 min at RT, spin at 250 x g and wash twice with 1 ml PBS



Perform Analysis within 24 hr

Vortex!

Data Evaluation

(2) Determining the percentage of Tetramer positive CD8+ T-lymphocytes: After setting a region on CD3+ T-lymphocytes (R5), the percentage of Tetramer+/CD8+ T-lymphocytes is determined in R6 in figure 1 (positive Tetramer) and figure 2 (negative Tetramer)

(Absolute Count of CD3+CD8+ cells) x (Tetramer-positive events as a percent of CD8+ cells / 100 = CD3+CD8+ Tetramer+ cells per ml whole blood

For more information please contact us

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