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Assessment of T-cell receptor repertoire by flow cytometry using the Beta Mark TCR Vβ repertoire kit and Kaluza analysis software

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IN THIS PAPER YOU WILL

- Discover a rapid and robust solution to investigate the TCR Vβ repertoire using flow cytometry
- Learn about a unique antibody combination and gating strategies for simultaneously assessing 24 different specificities of the TCR Vβ repertoire
- Understand how to visualize the results using the Comparison Plot in Kaluza software

Introduction/Background

T-cells play a central role in the immune system by recognizing and responding to non-self-antigens. T-cell receptors (TCRs) are located on the T-cell surface and are responsible for interacting with peptide fragments of antigens that bind to major histocompatibility complex (MHC) molecules. A great diversity of the T-cell receptor (TCR) repertoire—the range of different TCRs expressed—is a critical property of an effective immune system.

TCRs are highly diverse heterodimers, divided into two mutually exclusive populations. They are either a combination of α and β chains (expressed by most T-cells), or γ and δ chains (for 1 to 10% of T-cells). Similar to antibodies, TCRs are comprised of a variable and a constant region. The variable region, responsible for antigen recognition, is encoded by a number of variable (V) and joining (J) genes for the α and δ chains, and an additional diversity (D) gene for the β and γ chains. The VDJ segments are randomly attributed to each T-cell during their ontogeny in the thymus, and additional nucleotide addition/deletion at the junctions provides the TCR repertoire with its diversity.[1]

The analysis of TCR repertoire is valuable for better understanding the immune system, especially under different environments and stimulations, such as infections, immunodeficiencies, autoimmune diseases, and cancers studies (e.g., investigation of clonality), and contribute to discovering new therapeutic agents.[2] Several technologies and methods enable study of the TCR repertoire. A moderate- to high-resolution assessment is provided by molecular biology and PCR-based methods, such as TCR spectratyping and sequencing. These techniques, however, are expensive, time-consuming (requiring DNA isolation), relatively labor-intensive, and typically necessitate cell-sorting of highly pure T-cell populations. Therefore many researchers turn to flow cytometry.[3] Flow cytometry quickly measures the proportional TCR-Vβ usage in multiple T-cell subsets on a per-cell basis, without the need for cell-sorting.[4] In contrast to the semi-quantitative PCR method, flow cytometry antibodies detect the TCR proteins rather than measuring RNA levels. Combined with conjugated antibodies against other T-cell markers, it enables study of TCR expression on T-cell functional subsets (CD4+, CD8+, naïve, memory, etc.).[5]

The Beta Mark TCR Vβ Repertoire Kit (IM3497) has been designed for quantitative analysis of the TCR Vβ repertoire of human T lymphocytes by flow cytometric analysis, and has emerged as a reference for scientists studying this field.[1-10]

Beta Mark TCR Vβ Repertoire Kit – Composition and Principle

The Beta Mark TCR Vβ Repertoire Kit is composed of 8 vials containing mixtures of conjugated TCR Vβ antibodies corresponding to 24 different specificities representing about 70% coverage of normal human TCR Vβ repertoire. Table 1 shows the 24 antibody clones and their corresponding Vbeta segments according to the two main nomenclatures, from Wei, et al.[11] and the international ImMunoGeneTics (IMGT) system.[12]

Table 1: Kit antibody composition and associated Vbeta according to Wei, et al. and IMGT nomenclature

Tube	Clone	Conjugate	Vbeta (Wei et al.)	Vbeta (IMGT)
A	3D11 ZOE CH92	PE FITC + PE FITC	VB 5.3 VB 7.1 VB 3	TRBV5-5 TRBV4-1, TRBV4-2, TRBV4-3 TRBV28
B	FIN9 E17.5F3.15.13 TAMAYA1.2	PE FITC + PE FITC	VB 9 VB 17 VB 16	TRBV3-1 TRBV19 TRBV14
C	BA62.6 IMMU157 ELL1.4	PE FITC + PE FITC	VB 18 VB 5.1 VB 20	TRBV18 TRBV5-1 TRBV30
D	IMMU222 JU74.33 56C5.2	PE FITC + PE FITC	VB 13.1 VB 13.6 VB 8	TRBV6-5, TRBV6-6, TRBV6-9 TRBV6-6 TRBV12-3, TRBV12-4
E	36213 MPB2D5 VER2.32.1.1	PE FITC + PE FITC	VB 5.2 VB 2 VB 12	TRBV5-6 TRBV20-1 TRBV10-3
F	AF23 BL37.2 IG125	PE FITC + PE FITC	VB 23 VB 1 VB 21.3	TRBV13 TRBV9 TRBV11-2
G	C21 IMMU546 CAS1.1.3	PE FITC + PE FITC	VB 11 VB 22 VB 14	TRBV25-1 TRBV2 TRBV27
H	H132 WJF24 ZIZOU4	PE FITC + PE FITC	VB 13.2 VB 4 VB 7.2	TRBV6-2 TRBV29-1 TRBV4-3

As shown in Table 1, each tube contains 3 antibodies conjugated to 2 different fluorochromes, one TCR Vβ antibody is conjugated to FITC, another one to PE and the third to both FITC and PE. Therefore the TCR Vβ antibody conjugated to both FITC and PE shows up in the diagonal of the double positive region on an FITC vs. PE histogram (see example in Figure 1).

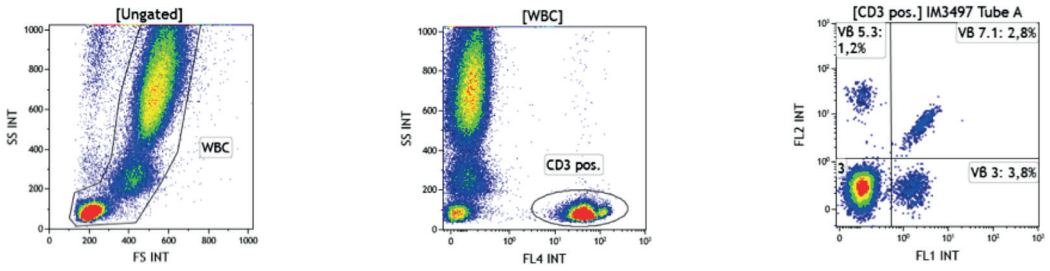

Figure 1: Example of data from tube A showing percentage of TCR VB5.3, VB7.1 and VB3 on T-cells using 3 antibodies and 2 colors

Antibody cocktails are ready to use; conjugated antibodies have been titrated to provide optimal staining performance on 100 µL of whole blood. For detailed protocol and methodology, please refer to the kit's instructions for use, available at beckman.com/techdocs.

Conjugated antibodies against other T-cell markers can be included in a multicolor flow cytometry panel to study TCR Vbeta expression in various T-cell subsets of interest, such as CD4 and CD8 for helper and cytotoxic T-cells, or CD45RA and CD45RO for naïve and memory T-cells. Additional conjugated antibodies against other TCR Vbeta not included in the kit may also be incorporated to increase the coverage of the repertoire.

Results

The Beta Mark TCR Vβ Repertoire Kit enables the assessment of 24 different TCR Vβ representing about 70% coverage of normal human TCR Vβ repertoire. It provides a snapshot of the diversity of the repertoire, percentage of each TCR Vβ among overall T-cells or specific subsets, and visibility to potential expansion of a specific clone.

Figure 2 provides an example of results obtained processing the 8 tubes with 24 conjugated antibodies on whole blood from a healthy donor. White blood cells are gated using the side and forward scatters and T-cells are gated using CD3. The three TCR Vβ of each tube are displayed in a dot plot, with the TCR Vβ conjugated to both FITC and PE appearing in the diagonal of the double positive quadrant.

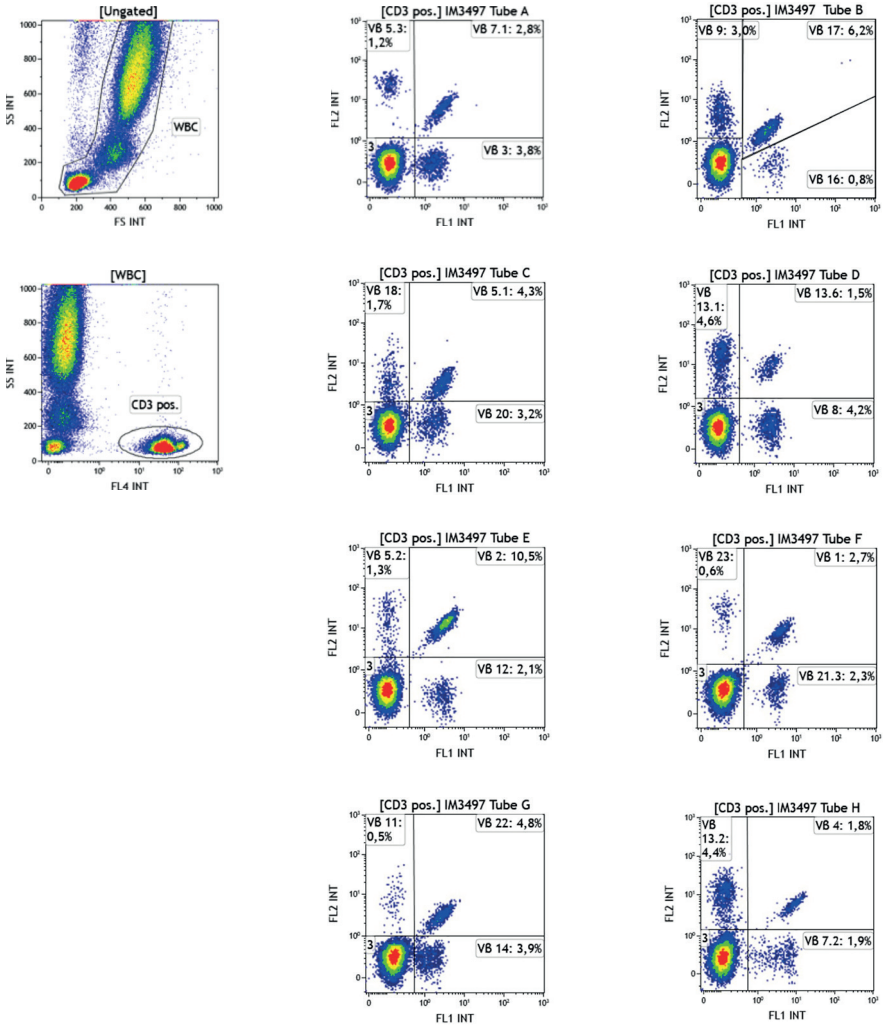

Figure 2: staining of healhy whole blood with the 24 conjugates of the Beta Mark TCR Vβ Repertoire Kit (gated on CD3+ T-cells) using Kaluza software

TCR Vβ expression varies across individuals. Polymorphisms have been described, and some blood samples may be negative for the expression of TCR Vβ 7.2[13] or Vβ 20.[14] Table 2 combines results obtained from the assessment of a cohort of 85 normal whole blood samples, and shows the mean percentage expression on T-cells (CD3+), helper T-cells (CD3+CD4+) and cytotoxic T-cells (CD3+CD8+), as well as the mean and maximum percentage expression, highlighting the great diversity among specimens.

Table 2: Mean percentages of expression of 24 TCR Vβ from a cohort of 85 normal specimens*

	CD3+					CD3+ CD4+					CD3+ CD8+				
Vbeta	mean	min	max	SD	CV	mean	min	max	SD	CV	mean	min	max	SD	CV
Vb1	3.53	1.89	11.7	1.35	0.38	3.32	1.62	14.2	1.5	0.45	4.24	1.4	8.21	1.62	0.38
Vb2	8.30	4.03	23.48	2.46	0.30	9.36	5.43	12.84	1.44	0.15	5.43	1.65	12.42	1.89	0.35
Vb3	4.68	0.52	15.71	3.13	0.67	4.37	0.66	10.04	2.29	0.52	4.44	0.32	13.80	3.13	0.70
Vb4*	1.91	0.79	3.26	0.48	0.25	2.03	1.20	2.83	0.37	0.18	1.90	0.61	4.34	0.80	0.42
Vb5.1	5.45	3.19	14.93	1.60	0.29	6.71	4.67	10.94	1.15	0.17	3.22	1.12	8.92	1.62	0.50
Vb5.2	1.33	0.49	4.98	0.52	0.39	1.33	0.5	2.87	0.38	0.28	1.12	0.18	3.53	0.57	0.51
Vb5.3	1.08	0.37	2.98	0.42	0.39	1.09	0.36	2.1	0.30	0.27	0.92	0.32	2.64	0.39	0.42
Vb7.1	2.56	0.64	20.01	2.08	0.81	1.93	0.59	3.8	0.62	0.32	3.39	0.87	7.14	1.23	0.36
Vb7.2	1.47	0.05	5.45	1.03	0.70	1.12	0.00	3.10	0.75	0.67	2.44	0.01	12.10	2.34	0.96
Vb8	4.68	2.36	29.47	2.94	0.63	4.81	2.94	6.73	0.77	0.16	4.06	0.86	11.43	2.06	0.51
Vb9	3.13	1.1	9.3	1.15	0.37	4.07	0.78	8.24	1.19	0.29	3.47	1.16	7.67	1.52	0.44
Vb11	1.04	0.25	5.11	0.62	0.60	0.87	0.3	1.9	0.26	0.30	0.92	0.14	2.25	0.46	0.50
Vb12	1.66	1	4.76	0.54	0.32	1.82	1.08	2.8	0.38	0.21	1.29	0.33	3.33	0.56	0.43
Vb13.1	3.83	1.62	8.16	1.06	0.28	4.03	1.93	7.7	0.98	0.24	3.42	0.41	5.35	0.91	0.26
Vb13.2*	2.80	0.80	5.28	1.23	0.44	2.81	0.72	7.27	1.18	0.42	3.34	0.96	9.62	1.80	0.54
Vb13.6	1.86	0.84	8.8	0.96	0.51	1.86	0.86	3.4	0.49	0.26	1.60	0.47	4.56	0.70	0.44
Vb14	3.49	1.33	8.03	1.36	0.39	2.59	1.57	4.68	0.65	0.25	5.74	1.5	14.3	2.55	0.44
Vb16	0.92	0.42	1.9	0.29	0.31	0.95	0.34	1.8	0.26	0.28	0.80	0.02	2.24	0.45	0.56
Vb17	5.15	2.28	12.61	1.28	0.25	5.46	3.12	8.32	1.02	0.19	5.06	1.83	11.18	1.97	0.39
Vb18	1.49	0.58	5.23	0.74	0.49	1.92	0.72	3.35	0.46	0.24	0.57	0.02	2.76	0.41	0.73
Vb20	2.52	0	9.73	1.38	0.55	2.60	0.04	5.3	1.12	0.43	2.31	0.08	5.61	1.50	0.65
Vb21.3	2.38	1.08	5.97	0.72	0.30	2.46	1.53	4.7	0.53	0.22	2.39	0.54	4.93	0.93	0.39
Vb22	3.84	1.99	9.89	1.17	0.31	4.26	1.98	8.48	1.11	0.26	3.17	0.54	6.47	1.19	0.38
Vb23	0.85	0.28	4.76	0.65	0.77	0.48	0.13	1.9	0.25	0.52	1.34	0.04	5.13	0.96	0.72
Total	69.95 (CD3+)					72.25 (CD3+ CD4+)					66.58 (CD3+ CD8+)				

Min: minimum value obtained; Max: maximum value obtained; SD: Standard Deviation; CV: Coefficient of Variation

* Except for tube H (Vβ4-Vβ7.2-Vβ13.2) which was tested on a different cohort of 46 normal specimens

The percentage values corresponding to the 24 Vβ specificities can be plotted in a bar chart to obtain a clonogram representation and improve data visualization. Figure 3 displays a clonogram representation of mean percentages and standard deviations of expression of 24 TCR Vβ from the cohort of 85 normal specimens from Table 2.

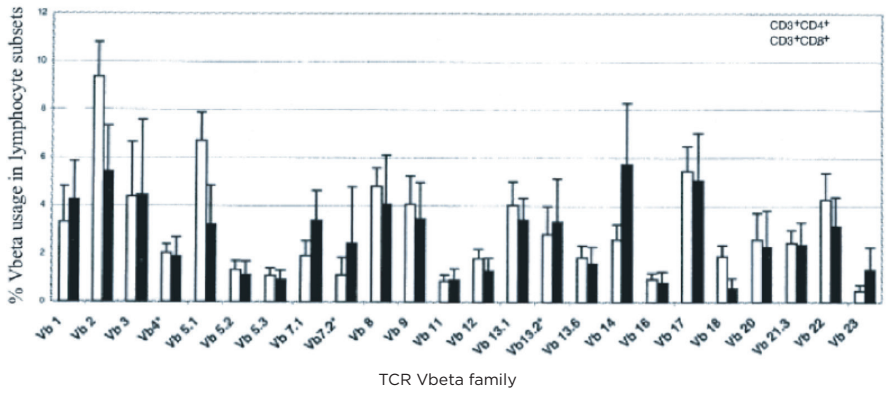

Figure 3: Clonogram representation of the TCR Vβ repertoire in CD4+ (open bars) and CD8+ (closed bars) CD3+ T-cell subsets

A Kaluza Composite is available that can be adjusted to use with data sets from any flow cytometer. To visualize the clonogram in Kaluza, the gating strategy shown in Figure 1 is applied to the acquired data files for all 8 samples in a Composite. A Comparison Plot is used to display the percentage of cells in the upper left, upper right and lower right quadrants of the FL1 vs. FL2 dot plot as three series in the plot.

Figure 4 shows a clonogram representation generated using the Kaluza Comparison Plot. Using Kaluza to display the clonogram streamlines the workflow and makes additional data analysis steps in a spreadsheet unnecessary.

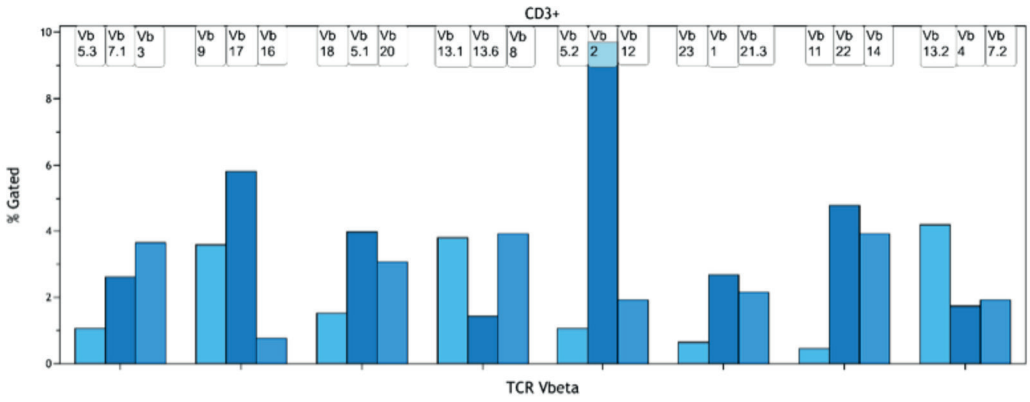

Figure 4: Clonogram representation of the TCR Vβ repertoire in CD3+ T-cells from a healthy whole blood

Conclusions

T-cells are the foundation of the immune response because they are able to respond to a wide variety of pathogens thanks to the high diversity of their TCR Vβ repertoire. Study of the TCR Vβ repertoire brings invaluable insights regarding T-cell response and overall mechanisms of the immune system, and has become a cornerstone in research related to various diseases, ranging from autoimmune disease to T-cell hematological malignancies.

Flow cytometry has emerged as a key technology for that purpose, thanks to several advantages over alternative methods such as molecular biology. The IOTest Beta Mark TCR Vβ Repertoire Kit has emerged as the reference to study the TCR Vbeta repertoire by flow cytometry. Its innovative concept—combining three mutually exclusive TCR Vbeta antibodies in two-color cocktails—enables reduction of the number of tubes, and improves research laboratory workflows.

The IOTest Beta Mark TCR Vβ Repertoire Kit contributed to democratizing the use of flow cytometry to assess the TCR Vβ repertoire, providing laboratories with the opportunity to perform in-depth analysis with only 3-color flow cytometry (FITC, PE + a third color for a gating marker), while providing more advanced flow cytometrists with the possibility to run more complex experiments by adding drop-in markers to investigate T-cell subsets.

By using Kaluza analysis to display the clonogram, the data analysis workflow for assessing the TCR Vβ repertoire can be streamlined to only a single software package without the need for exporting statistical results and further processing in spreadsheets. The available example file can be modified for use with flow cytometry data from any instrument, and the gating strategy can be adjusted to add further drop-in markers.

References

1. Estimating T-cell repertoire diversity: limitations of classical estimators and a new approach. Laydon et al. Phil. Trans. R. Soc. B 370: 20140291. 2015

2. History, applications, and challenges of immune repertoire research. Cell Biology and Toxicology. Liu et al. Cell Biol Toxicol 34:441–457. 2018

3. Bias in the αβ T-cell repertoire: implications for disease pathogenesis and vaccination. Miles et al. Immunol Cell Biol. Mar; 89(3):375-87. 2011

4. Quantifying Distribution of Flow Cytometric TCR-Vβ Usage with Economic Statistics. M. van der Geest et al. PLoS One. 10(4): e0125373. 2015

5. Overview of methodologies for T-cell receptor repertoire analysis. Rosati et al. BMC Biotechnology volume 17, Article number: 61 2017

6. Rapid analysis of the Vbeta repertoire of CD4 and CD8 T lymphocytes in whole blood. MacIsaac et al. Journal of Immunological Methods 283 9 – 15. 2003

7. Flow Cytometric Assessment of TCR-Vβ Expression in the Evaluation of Peripheral Blood Involvement by T-Cell Lymphoproliferative Disorders. Morice et al. Am J Clin Pathol; 121:373-383. 2004

8. Flow cytometric assessment of TCR-VBETA expression in the evaluation of peripheral blood involvement by mature T-cell leukaemias. Elisabeth Todorova. Pathology. Volume 41, Supplement 1, Page 86. 2009

9. Superantigens and Chronic Rhinosinusitis: Skewing of T-Cell Receptor Vβ-Distributions in Polyp-Derived CD4+ and CD8+ T-cells. Conley et al. American Journal of Rhinology, Volume: 20 issue: 5, page(s): 534-539. 2006

10. TCR Analyses of Two Vast and Shared Melanoma Antigen-Specific T-cell Repertoires: Common and Specific Features. Simon et al. Front. Immunol., 30 August 2018

11. The extent of the human germline T-cell receptor Vβ gene segment repertoire. Wei et al. Immunogenetics, 40, 27-36. 1994

12. http://www.imgt.org/

13. A genetically determined insertion/deletion related polymorphism in human T-cell receptor β chain (TCRB) includes functional variable gene segments. Zhao et al. J. Exp. Med., 180, 1405- 1414. 1994

14. Effect of TcRBV6S1 and BV20S1 DNA polymorphism on their expression level. Charmley et al. The Immunologist, 4, 26-28. 1996

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FLOW-6377APP02.20