TECHNICAL NOTE

Accurate Detection of T Clonality: Importance of TRBC1 and TRBC2 in Flow Cytometry

Context and Challenge in T Cell Clonality

The evaluation of T lymphocyte clonality is essential in the haematological diagnosis of T-cell neoplasms.

Traditionally, this analysis was performed using molecular TCR rearrangement techniques; however, flow cytometry has incorporated new protein markers that allow clonality to be detected quickly and specifically. A key advance was the introduction of the TRBC1 monoclonal antibody directed against the β1 constant chain of the TCR receptor.

Recent studies have shown that the detection of TRBC1 monotypic populations indicates the presence of a malignant T clone with high sensitivity[1]. Berg et al. reported that a single TRBC1 antibody was able to identify clonality in 100% of the T-cell lymphoma cases analysed, proposing this technique as a robust and routine assay in cytometry [1]. However, what about clones that do not express TRBC1? This is where the need for a second complementary marker arises.

Limitations of the Exclusive Use of TRBC1

Although TRBC1 has proven to be extremely useful, relying solely on this marker can lead to inconclusive or even false negative results.

Approximately 30–40% of clonal T cells do not express TRBC1, as they use the constant β2 chain (TRBC2) instead [2,3]. In these cases, the neoplastic population will appear TRBC1-negative by cytometry, and it may be difficult to distinguish it from a normal polyclonal profile if precautions are not taken. It has been documented that simple staining with TRBC1 can generate spurious TRBC1-dim subpopulations, which confuses diagnostic interpretation [3].

All these artefacts are resolved by incorporating an anti-TRBC2 antibody into the panel. In other words, a TRBC1-negative T clone could go unnoticed or be misclassified as polyclonal if TRBC2 is not used in parallel. Ferrari et al. emphasised this reality when developing targeted therapies, highlighting that to effectively address T-cell lymphomas, it is necessary to consider both TRBC1 and TRBC2 isoforms, as malignant cells can utilise either one [2].

Advantages of Combined TRBC1 + TRBC2 Detection

The combined use of TRBC1 and TRBC2 in flow cytometry allows for comprehensive clonality assessment, covering 100% of the TCR β repertoire of T lymphocytes [1,3]. Just as in B cell immunophenotyping, both κ/λ light chains are analysed to confirm monoclonality, in T cells, double labelling with TRBC1/TRBC2 ensures that no clone will go undetected [3]. Horna et al. (2021) highlighted that, although TRBC1 alone is usually sufficient to detect TCR restriction in many cases, having an anti-TRBC2 anti e "completes the picture" and facilitates κ/λ-style analysis in T populations. The combination of both antibodies offers greater diagnostic accuracy, avoiding the doubts that a single stain can generate. Specifically, the TRBC1+TRBC2 duality allows for:

- **Identification of the T clone in 100% of scenarios:** any neoplastic population Will express one of the two TCR β constants. With TRBC1+TRBC2, a clonal population will show complete positive staining for one and negative for the other, achieving unequivocal identification[1,3].
- **Reduction of ambiguous results:** double labelling eliminates atypical fluorescence patterns (e.g., weak TRBC1 cells) that could be misinterpreted with a single marker[3]. This reduces the number of "suspicious" cases that previously required additional molecular analysis for confirmation.
- **Simplified and rapid workflow:** obtaining a clear clonality result in cytometry speeds up diagnosis and, in many cases, eliminates the need for TCR rearrangement PCR testing, saving time and resources[1].

In summary, combining TRBC1 and TRBC2 maximises sensitivity and certainty in the detection of pathological T clones. This strategy is already recognised as best practice in multiparametric cytometry panels for T-cell leukaemias/lymphomas, in line with the latest recommendations in the field.

Performance of Optimal Conjugates (TRBC2 – Immunostep)

With the commercial availability of anti-TRBC2 antibodies (such as the SAM.2.rMAb clone developed by Immunostep), laboratories can easily implement dual detection. It is important to select high-intensity fluorochrome conjugates that ensure clear separation between TRBC2-positive and TRBC2-negative populations. In a recent technical report (November 2025) from the General Cytometry Service (University of Salamanca), the performance of the TRBC2 antibody (SAM.2.rMAb) conjugated with different fluorochromes in normal peripheral blood was evaluated.

The results showed that the Super Bright Violet 790 (SBV790) format offered a high staining index in both CD4+ and CD8+ T lymphocytes, outperforming other violet conjugates tested. In other words, TRBC2–SBV790 provided excellent resolution between TRBC2-positive (TRBC2 clonal) and negative cells, supporting its practical utility for discriminating populations in cytometry.

For example, in CD4+ T cells, an IS ≈42 was obtained with SBV790 (compared to IS ≈23 with SBV610 or ≈10 with SBV710), and in CD8+ T cells, an IS ≈21 (compared to much lower values with weaker conjugates).

This performance makes TRBC2–SBV790 a recommended option when high-sensitivity violet laser channels are required (Fig. 1).

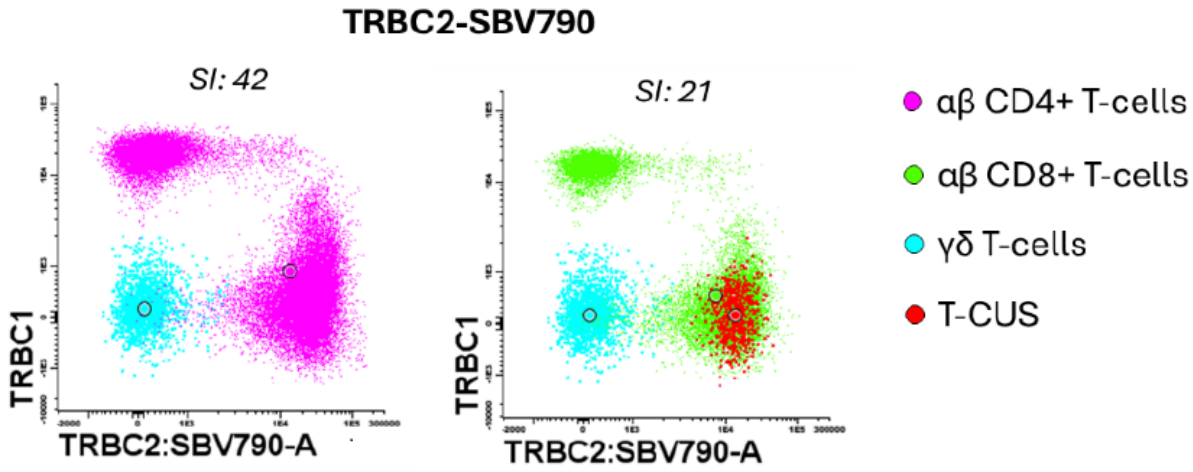

Figure 1: Excellent resolution of anti-TRBC2 (SAM.2.rMAb) conjugated to SBV790. Bivariate diagrams of TRBC1 vs TRBC2-SBV790 in human peripheral blood show a clear separation between positive and negative populations. The SBV790 conjugate has a high staining index in both CD4+ (SI = 42) and CD8+ (SI = 21) T lymphocytes, allowing robust identification of TRBC2-positive populations when used in combination with TRBC1.

Additionally, the PE format of the TRBC2 antibody demonstrated the highest signal intensity among all conjugates evaluated, serving as an optimal reference. In the aforementioned evaluation, TRBC2–PE achieved extraordinary staining indices (e.g., SI ≈128 in CD4+ T cells) thanks to its brightness, ensuring a very clear separation.

This is especially useful for detecting minority clones, as a bright fluorochrome highlights even small populations of positive cells. Below is the promotional image for Immunostep's TRBC2 (PE format) product, illustrating its high performance in separating TCRβ2-positive and -negative populations:

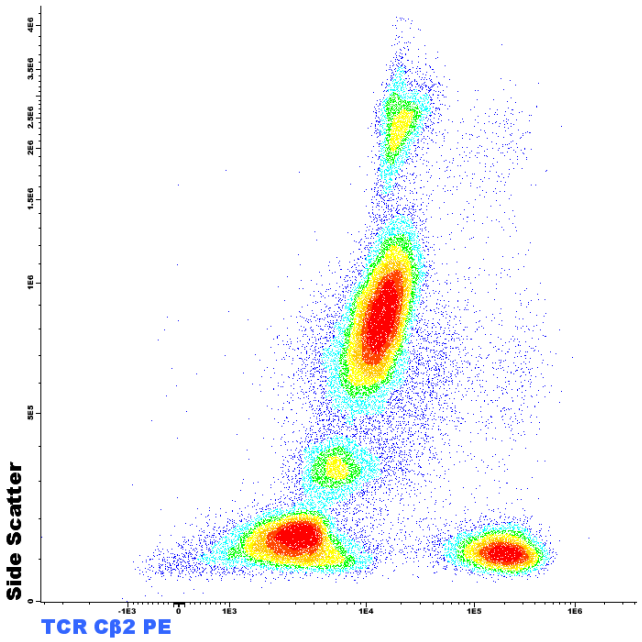

Figure 2: Graphical representation of the performance of the anti-TRBC2 antibody in PE format (clone SAM.2.rMAb). The intense fluorescence obtained with TRBC2–PE is evident, allowing TRBC2-positive cells (monoclonal TCRβ2 population) to be clearly distinguished from TRBC2-negative cells within a polyclonal sample. In comparative studies, this PE conjugate showed greater intensity and signal-to-background ratio than other reagents on the market, facilitating more accurate T-clone identification.

Conclusions and Recommendations

In a clinical setting where every immunophenotypic detail counts, integrating both TRBC1 and TRBC2 markers into flow cytometry panels provides superior diagnostic confidence .

Scientific evidence supports that this pair of antibodies detects T-cell cloning with maximum accuracy, avoiding omissions of TRBC2 clones that may go unnoticed if only

TRBC1 is used. Therefore, clinical laboratories, hospitals, and research centres dedicated to haematological diagnosis should strongly consider incorporating both TRBC1 and TRBC2 into their protocols. [1-3]

Immunostep has developed optimal conjugates (such as TRBC1–FITC, PE-Cy7, TRBC2–PE, TRBC2–SBV790, among others) that can be easily integrated into standard multiparametric panels. Thanks to their high staining index and specificity, these reagents simplify the detection of T-cell clonality at the routine level, bringing this analysis on par with the already established κ/λ study in B-cell populations.

We invite your laboratory to update your T-cell immunophenotype panels by incorporating TRBC1 + TRBC2: this minimal investment in reagents can translate into faster, more complete and reliable diagnoses for T-cell leukaemias and lymphomas. Ultimately, the combined use of TRBC1 and TRBC2, supported by state-of-the-art bright conjugates, ensures that no pathological clone will go undetected, improving the quality of the diagnostic service offered to your patients and researchers. Take advantage of this innovation and take the accuracy of your flow cytometry to the next level!

References

[1] Berg H., Otteson G.E., Corley H., et al. Flow cytometric evaluation of TRBC1 expression in tissue specimens and body fluids is a novel and specific method for assessment of T-cell clonality and diagnosis of T-cell neoplasms. Cytometry B Clinical Cytometry. 2021; 100(3):361–369.

[2] Ferrari M., Baldan V., Ghongane P., et al. Targeting TRBC1 and TRBC2 for the treatment of T cell lymphomas. Cancer Research. 2020; 80:2183.

[3] Horna P., Shi M., Olteanu H., Johansson U. Emerging role of T-cell receptor constant β chain-1 (TRBC1) expression in the Flow cytometric diagnosis of T-cell malignancies. International Journal of Molecular Sciences. 2021; 22(4):1817.