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Simplifying Progress

Streamlining Immune Cell Profiling: Advanced Panel Design with Sartorius Antibodies and New iQue® HTS Platform

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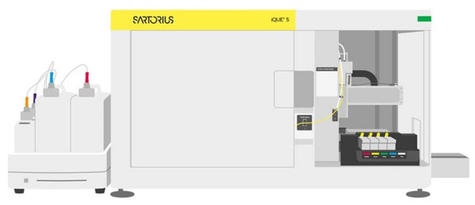
Introduction

- As interest in CAR-T cells and immune-based oncology therapies increases, there is an increasing requirement for efficient and rapid phenotyping of potential cell therapy products.
- To address this demand, it is essential that both the instruments for cell interrogation and the supporting tools for panel design can flexibly accommodate the variety of required markers.
- This study highlights the application of Sartorius selected antibodies in conjunction with the Sartorius panel design tool to streamline the assay building process.
- When integrated with the next generation iQue® HTS cytometer platform this provides enhanced flexibility and expanded channel coverage.
- To showcase this workflow, data was generated for a T cell phenotyping panel and tested in PBMCs.

New iQue® 5 HTS platform

The new, next generation iQue® instrument builds on the established features of the existing iQue® 3 platform to enhance the customer experience and support extra flexibility.

Equipped with adaptable automation capabilities, it facilitates seamless integration with automation solutions, enhancing high-throughput screening and minimizing the need for manual intervention.



- Accelerated market-leading speed with use of the patented air gap technology to deliver 384-well sampling in <20 min.
- Improved Clog detection and air-gap sensor for monitoring.
- Set individual channel gains with SiPM.
- Improved usability, fluidics and data analytics including higher volume acquired in less time.
- Up to 27 channel capacity improving flexible workflow support for easier panel design.

Emission Filter	Laser			
	405 nm	488 nm	561 nm	640 nm
445/45 nm	✓ Pacific Blue			
525/45 nm	✓ BV510	✓ FITC		
586/20 nm	✓ BV605	✓ EYFP	✓ PE	
615/20 nm	✓ Qdot 605	✓ PI	✓ PE-Dazzel594	
667/30 nm	✓ BV650	✓ PerCP	✓ PE-Cy5	✓ APC
695/40 nm	✓ BV711	✓ PerCP-Cy5.5	✓ PE-Cy5.5	✓ AF680
725/40 nm	✓ BV750	✓ PerCP-eFluor7	✓ PE-AF700	✓ AF700
780/60 nm	✓ BV786	✓ PE-Cy7	✓ PE-Cy7	✓ APC-Cy7

Table 1: Laser and channel details for iQue® 5 Platform

T Cell Panel Design

A comprehensive T cell panel was developed using the Sartorius panel tool (table 1 below).

- Two panels were designed for use on either iQue® 3 or 5 using a base panel covering CD3, CD4, CD8 and a viability marker.
- Further markers for activation (CD69, CD25), exhaustion (LAG-3, TIM-3, PD-1) or memory (CCR7, CD62L), were added for further characterization.
- Using the new iQue® 5 HTS platform a further 4 markers were incorporated to enable the capture of 11 data channels, along with forward and side scatter, from a 96-well plate.
- The addition of a fourth laser increases the flexibility for panel design and ability to capture more data.

Laser	Channel	Target	Fluorochrome	iQue®3 panel
Violet 405	V445/45	Viability	Zombie Violet	VL1 – 445/45
Violet 405	V525/45	CD8	BV510	VL2 – 530/30
Violet 405	V615/20	CD4	BV605	VL4 – 615/24
Violet 405	V780/60	CD69	BV786	
Blue 488	B525/45	CD62L	FITC	
Yellow 561	Y586/20	CD25	PE	BL2 – 572/28
Yellow 561	Y615/20	CCR7	PD-Dazzle594	
Yellow 561	Y780/60	PD-1	PECy7	BL5 – 780/60
Red 640	R667/30	LAG-3	AF647	RL1 – 675/30
Red 640	R695/30	TIM-3	APC	
Red 640	R780/60	CD3	APC-Fire750	RL2 - 780/60

Table 2: Predicted panel layout

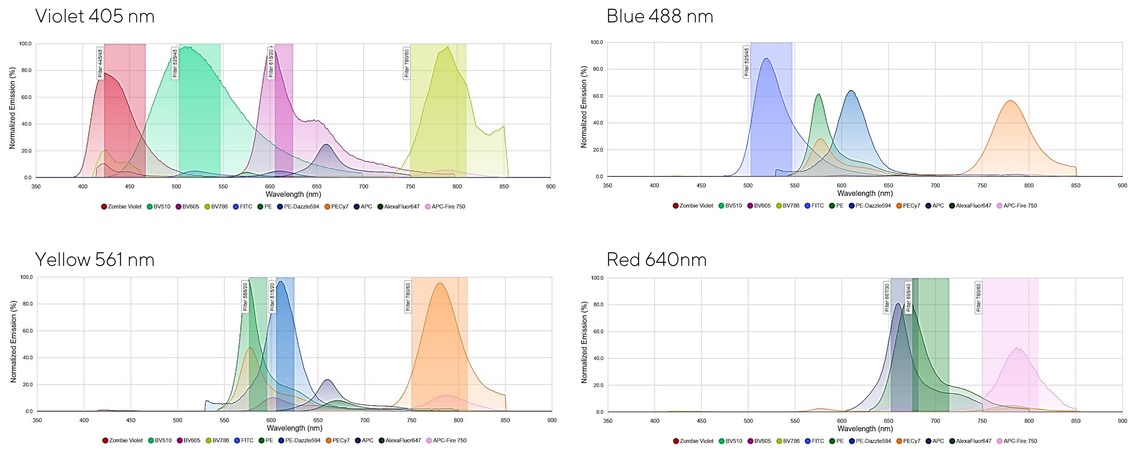


Figure 1: Predicted panel layout across lasers for iQue® 5 Platform, note overlapping spectra for APC and AF647

Compensation Assessment

Initially, compensation particles were tested with the selected antibodies to automatically calculate compensation spillover values using iQue Forecyt® software, before applying the panel to immune cells.

- In iQue® 5, PE conjugated antibodies can use the yellow laser excitation for improved resolution.
- Spillover matrix highlights potential issue with TIM-3 and LAG-3 due to overlapping spectra.
- iQue Forecyt® compensation allows overlapping fluorophores to be isolated and successfully analyzed.

iQue® 3 – 7 markers

Spillover Matrix

Primary Channel	Spillover Channel	CD25 (BL2-H)	PD-1 (BL5-H)	LAG-3 (RL1-H)	CD3 (RL2-H)	V/Blue MI (VL1-H)	CD8 (VL2-H)	CD4 (VL4-H)
CD25 (BL2-H)			0.64	0.02	0.08	0.04	0.53	7.35
PD-1 (BL5-H)		1.81		0.03	16.50	0.06	0.36	0.26
LAG-3 (RL1-H)		0.01	0.07		6.27	0.03	0.12	0.05
CD3 (RL2-H)		0.06	1.36	5.67		0.08	0.32	0.21
V/Blue MI (VL1-H)		0.00	0.00	0.00	0.00		75.61	2.74
CD8 (VL2-H)		0.06	0.02	0.01	0.02	2.31		16.41
CD4 (VL4-H)		0.47	0.20	0.08	0.03	2.08	0.67	

iQue® 5 - 11 markers

Spillover Matrix

Primary Channel	Spillover Channel	CD62L (B525-H)	TIM-3 (R667-H)	LAG-3 (R695-H)	CD3 (R780-H)	V/Blue MI (V445-H)	CD8 (V525-H)	CD4 (V615-H)	CD69 (V780-H)	CD25 (Y586-H)	CCR7 (Y615-H)	Y667-H	PD-1 (Y780-H)
CD62L (B525-H)			0.02	0.00	0.02	0.18	3.99	0.36	0.04	0.11	0.23	0.00	0.16
TIM-3 (R667-H)		0.02		13.50	11.39	0.09	0.13	0.15	0.75	0.23	0.88	0.00	5.00
LAG-3 (R695-H)		0.09	399.11		61.97	0.60	0.42	0.19	0.32	0.45	0.97	0.00	12.77
CD3 (R780-H)		0.03	3.01	0.46		0.11	0.10	0.06	6.43	0.13	0.37	0.00	35.94
V/Blue MI (V445-H)		0.21	0.05	0.02	0.02		19.84	0.70	0.04	0.06	0.06	0.00	0.04
CD8 (V525-H)		0.48	0.00	0.00	0.01	13.13		20.70	1.57	0.29	0.73	0.00	0.08
CD4 (V615-H)		0.18	0.42	0.08	0.05	19.58	1.75		8.39	49.72	72.33	0.00	7.71
CD69 (V780-H)		0.11	0.13	0.06	18.87	17.59	1.61	0.30		0.18	0.33	0.00	5.42
CD25 (Y586-H)		1.58	0.01	0.00	0.01	0.09	0.28	4.99	0.13		24.52	0.00	0.93
CCR7 (Y615-H)		0.67	0.41	0.10	0.24	0.11	0.16	14.73	0.69	34.80		0.00	5.94
Y667-H		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00
PD-1 (Y780-H)		0.24	0.01	0.01	9.88	0.05	0.06	0.05	6.22	2.07	0.69	0.00	

Table 3: Spillover Matrix generated in iQue® Forecyt Software on both iQue®3 and iQue®5.

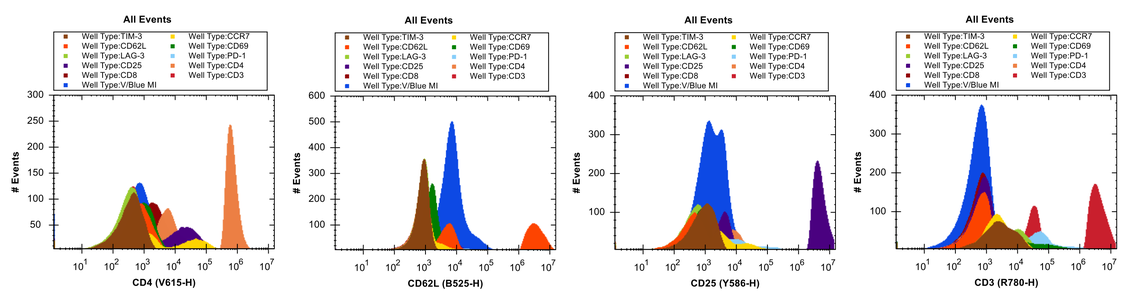


Figure 2: Example compensation plots across lasers, demonstrating clear positive peak in selected channel.

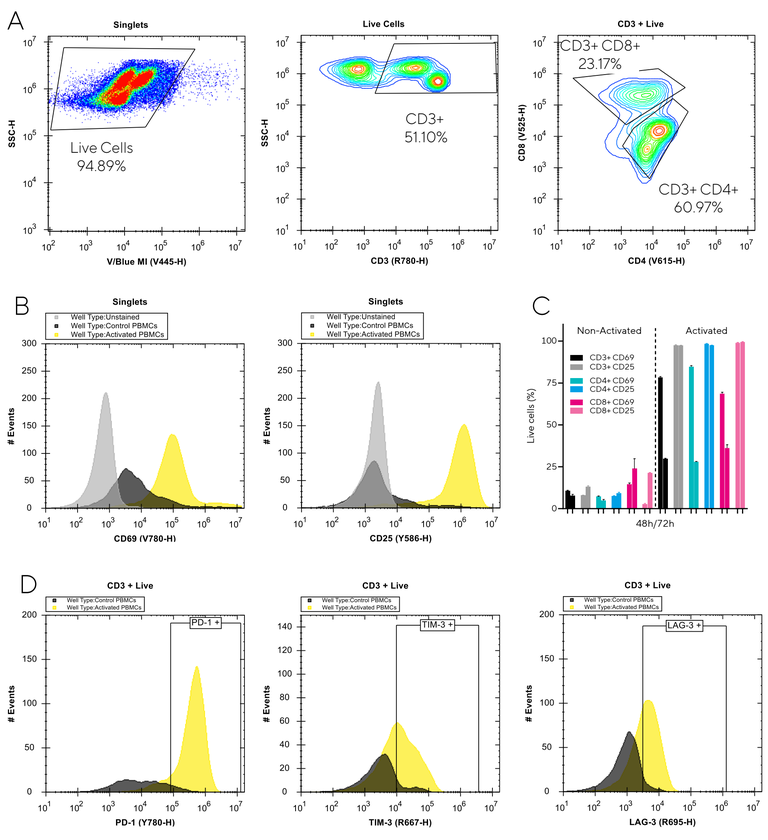
Phenotype Profiling in PBMCs

Human PBMCs were cultured in RPMI 1640 media with 10% serum and IL-2, with or without CD3/CD28 T cell activation beads (1 bead per cell) to activate the T cell population. Over the following 72 hours, the cells were assessed using the panel.

Cells were assessed using the following strategy:

- All events to detect cells (FSC Vs SSC) and singlets (not shown)
- Viability marker for live cells
- CD3+ cells
- CD4+ and CD8+ populations

→ Activation – CD69, CD25
→ Exhaustion – PD-1, LAG-3, TIM-3
→ Memory – CCR7, CD62L



- A - Live, T cell populations at 48 h.
- B - Activation markers, plots show treatment groups for all cell events to highlight activated population.
- C - Bar graph compares activation at 48 and 72 h for each T cell population.
- Clearly see CD69, early marker for activation reducing by 72 h while CD25 remains high.
- D – Exhaustion marker plots show data for the live CD3+ population. These markers are known to show a transient increase after activation and at 48 h all three show some increase but unlikely to be true exhaustion.
- E - Displays potential memory phenotypes. Using CCR7 and CD62L it is possible to define two groups Tscm/Tcm (less differentiated) and Tem/Teff (more differentiated) populations. Post activation the CD3+ T cells have moved to more differentiated state (control 16%, activation 76%).

Conclusion

- In summary, the new iQue® 5 HTS platform provides several enhancements to support users, including increased flexibility in designing complex panels.
- The integration of Sartorius Antibodies, the panel design tool and the iQue® 5 HTS platform offers a streamlined approach for creating complex panels to profile immune cell populations.
- The panel can be expanded and further enhanced by incorporating cytokine profiling using the iQue Qbead® portfolio, leveraging the iQue® Platform's capacity to independently track both cell and bead populations within the same sample.