

# Liquid Biopsy Based Monitoring of PD-L1 Expression in Non-Small Cell Lung Cancer (NSCLC) Patients for Immunotherapy

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Poster No: OTH03

## Introduction

Discovery of the role played by PD-L1 to advanced stage NSCLC and the development of therapeutics targeting PD-1 and PD-L1 has put forward immunotherapy as a promising technique for cancer management. PD-L1 helps cancer cells evade immune surveillance by binding to its PD-1 immune modulatory receptor on the surface of activated T-cells and thereby deactivating T-cells cytotoxic activity. Periodic monitoring of PD-L1 expression via immunofluorescence technique in liquid biopsy samples can be a significant tool to assist in administering immunotherapy. This is challenging as the tumor samples are contaminated with other cell types that interfere with PD-L1 analysis. We used Clearbridge BioMedics' ClearCell® FX, a label free microfluidic platform to deplete these contaminants to provide ready-to-analyze samples and Leica Biosystems' BOND RX for fully automated immunofluorescence staining. Here, we showcase a minimally invasive and automated workflow tested and proven on clinical samples using ClearCell® FX and BOND RX for immunofluorescence based PD-L1 analysis.

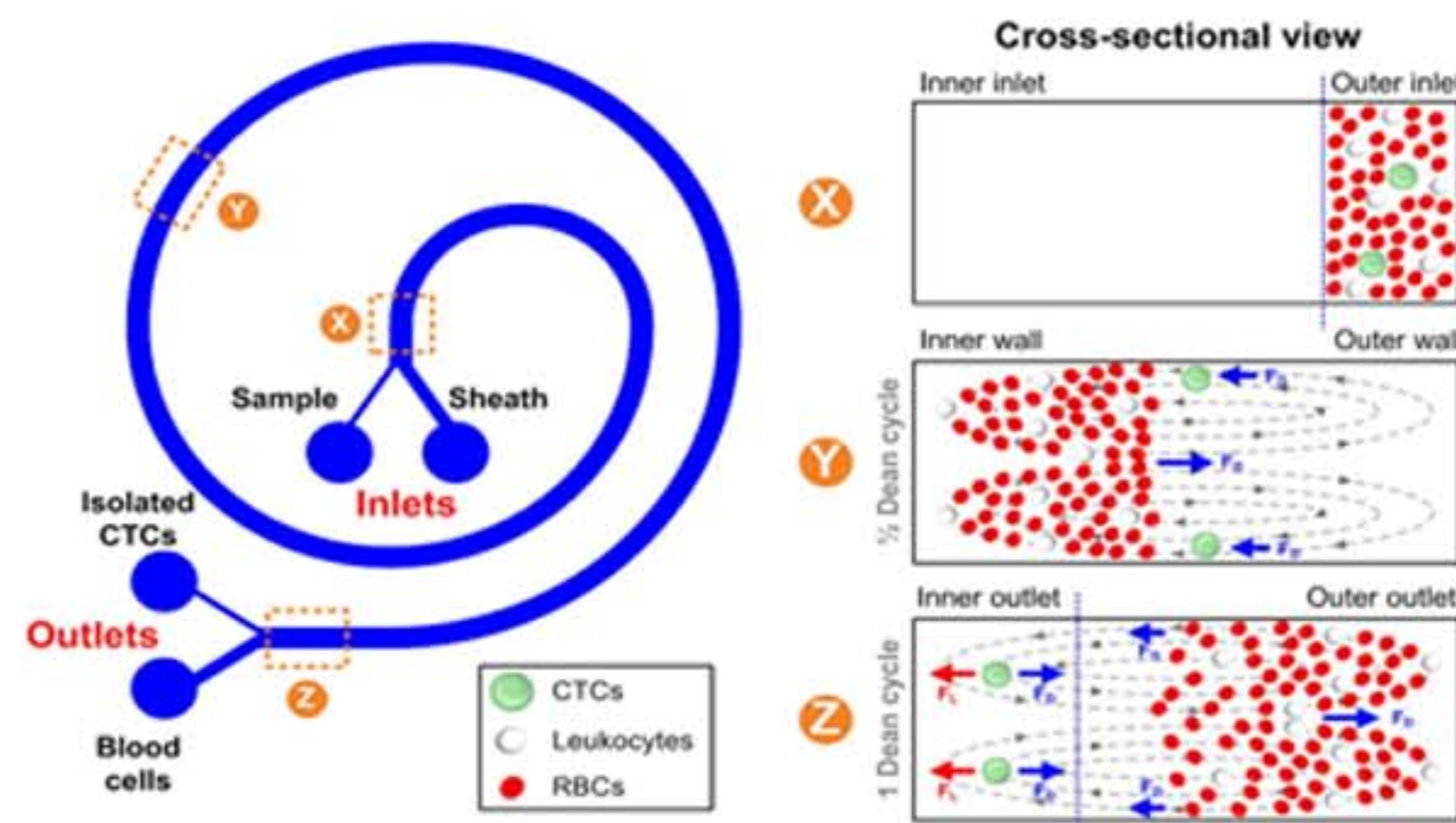
## CTC Enrichment by ClearCell® FX



- Label-free isolation method
- Enrichment of CTCs based on size & inertia
- Retrieval of wholly *intact* and *viable* cells
- High purity,  $5\log_{10}$  depletion of WBCs
- Process large blood volume of 7.5ml
- Fully automated CTC enrichment platform
- Small footprint

ClearCell® FX enriches for CTCs in a label-free approach that allows for high purity CTC isolation. The technology utilizes an inertial focusing principle generated within a radial microfluidic chip to subject CTCs and other cell types to both inertial and Dean drag forces to separate CTCs from smaller cells in the blood.

## Isolation by Size



- Smaller hematologic cells [red blood cells (RBCs) ~8µm; leukocytes ~8-15µm] are affected by the Dean Drag and migrate to outer wall.
- Larger CTCs (~15-20µm) experience strong inertial lift forces as indicated by the red arrows and is focused along the microchannel inner wall.

## Results

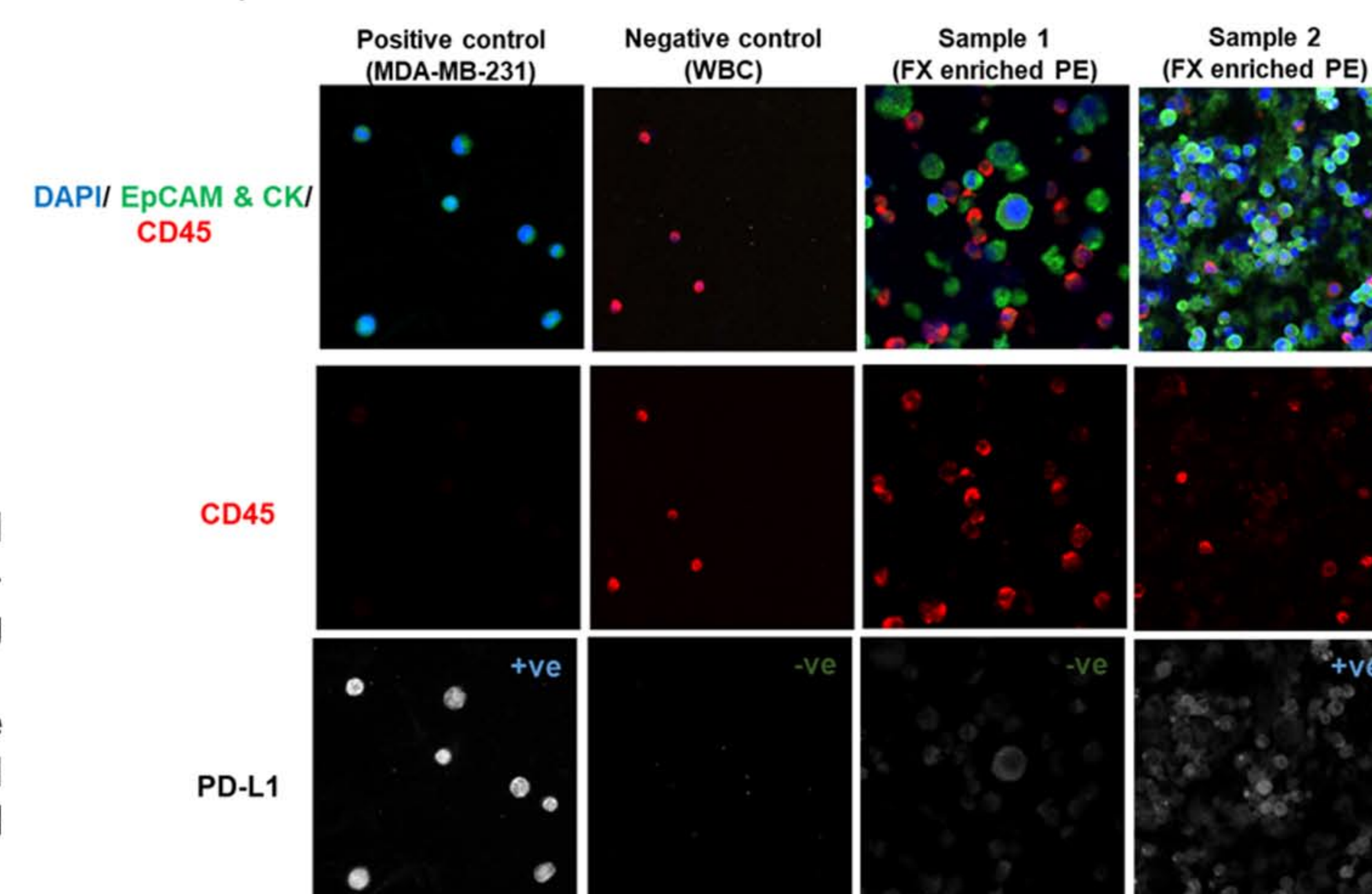
- Minimal cell losses (<5%) on control samples

	Cell loss %
Run 1	4
Run 2	3
Run 3	4.5
Run 4	3.5

- Good staining quality with high signal to noise ratio (SNR) (>4.5)

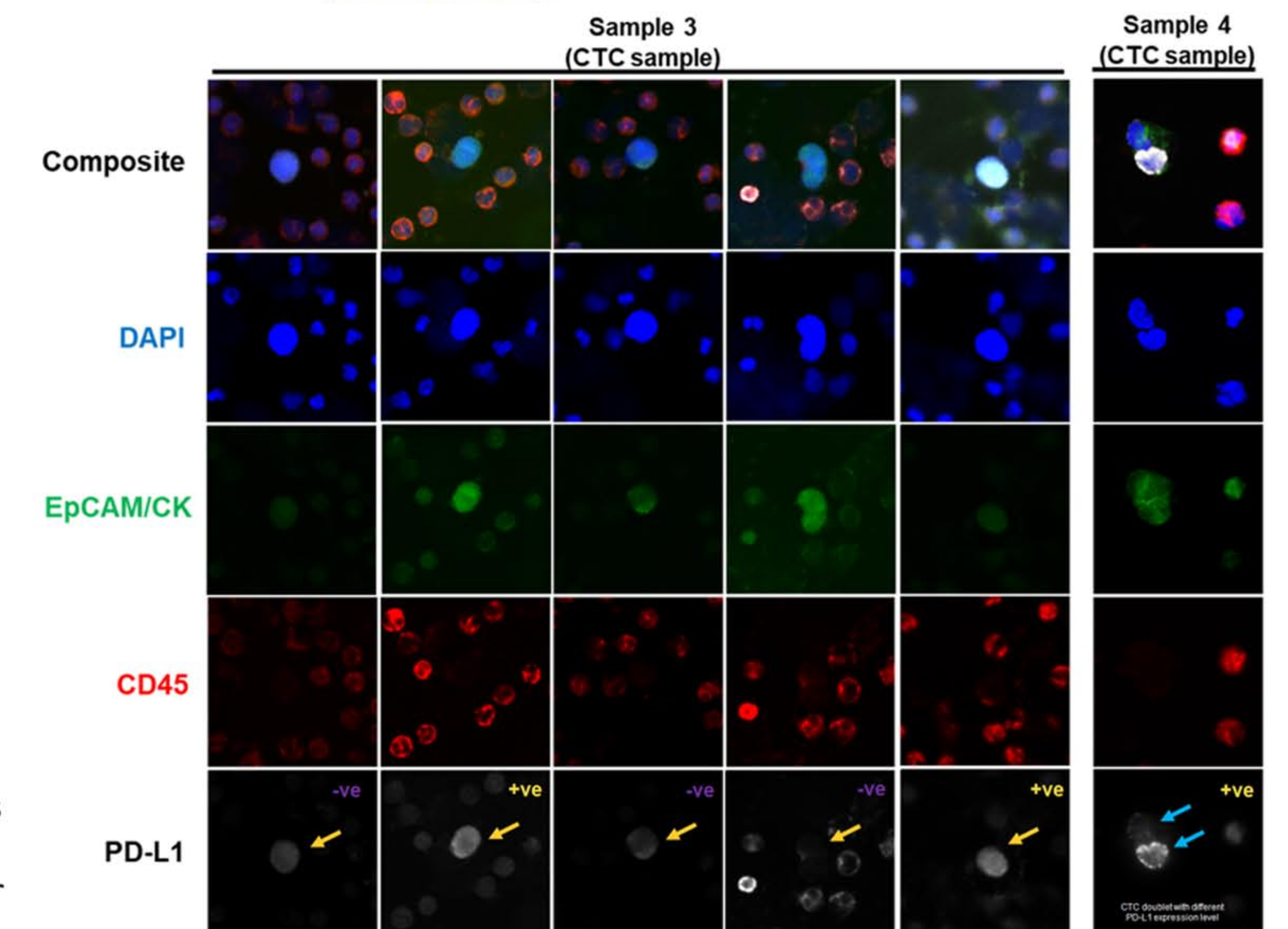
	Epithelial SNR	CD45 SNR
Run 1	5.36	4.58
Run 2	6.58	5.71
Run 3	8.17	5.15
Run 4	8.96	6.61

- Among the two pleural effusion samples analyzed, tumor cells expressed strong level of epithelial markers. However, PD-L1 positivity were variable, and detected on 7.6% and 71.8% of tumor cells respectively. Control slides were included in each cycle. Empirical threshold of the assay was determined using positive (MDA-MB-231 cells) and negative controls (white blood cells from healthy individuals), with at least 75 cells scored in each control sample

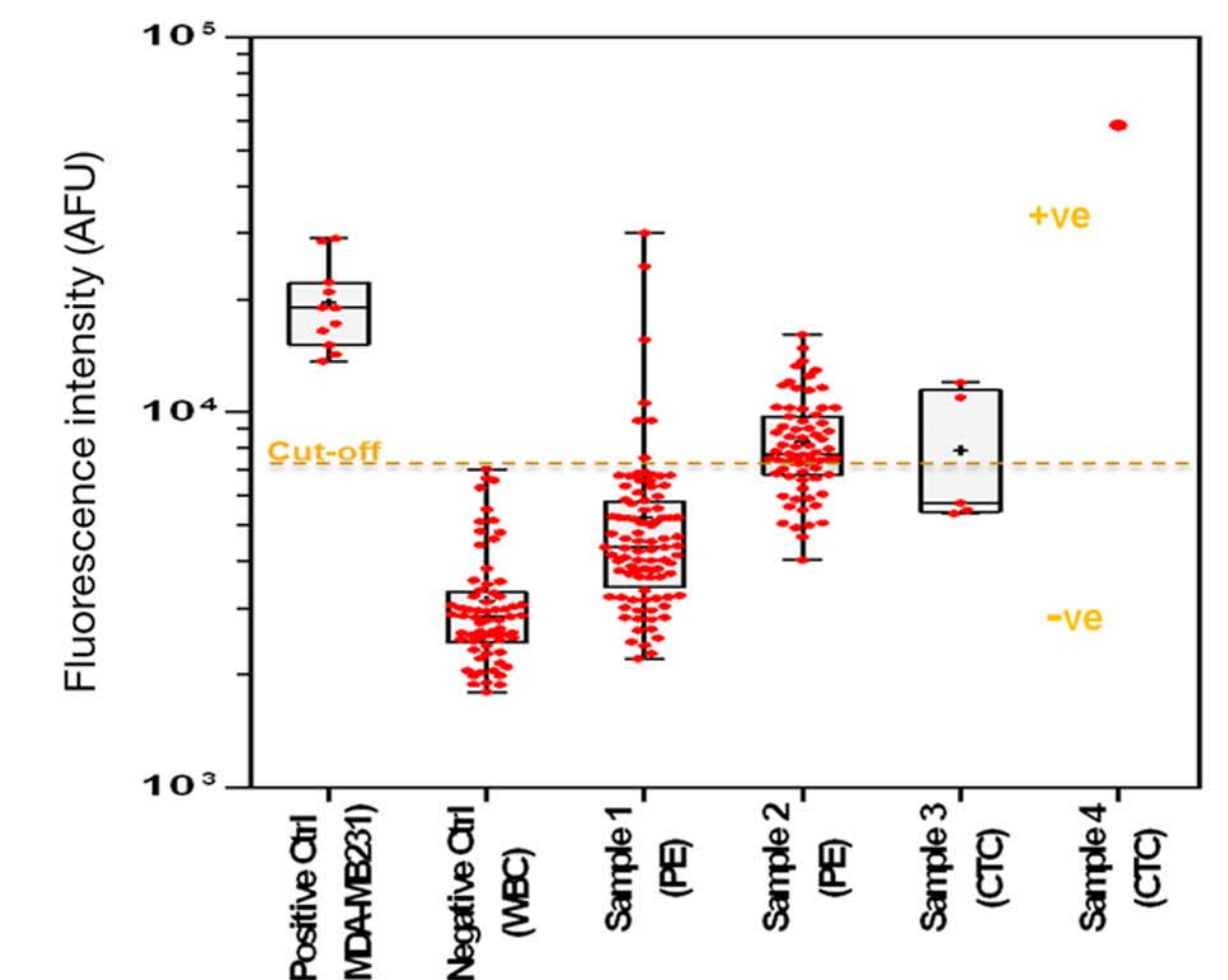


Abbreviation: FX- ClearCell® FX enrichment system, PE- Pleural Effusion, WBC- white blood cells

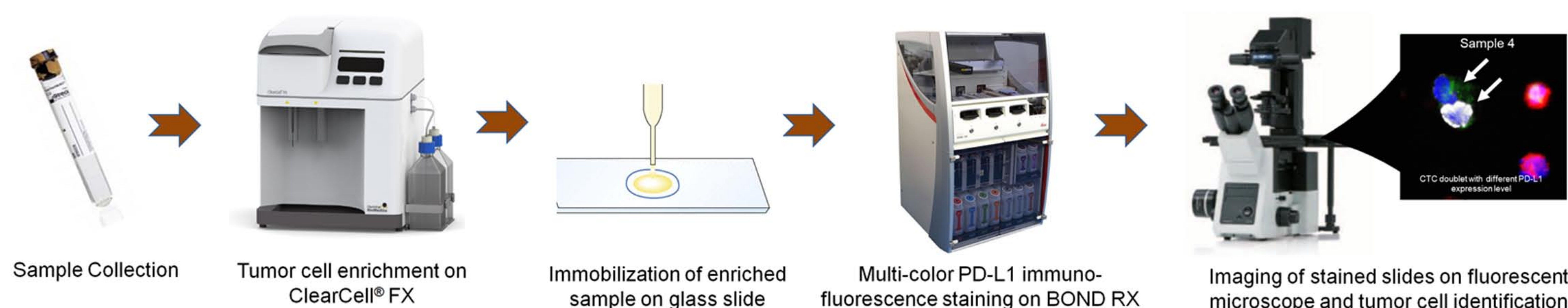
- Isolated CTCs expressed variable level of both epithelial markers (EpCAM/Cytokeratin) and PD-L1 (yellow arrows)
- PD-L1 positivity were 40% (2/5 CTCs) and 100% (1/1 CTCs) among the two peripheral blood samples analyzed
- Interestingly, a CTC "doublet" isolated from one of the peripheral blood samples had a cell with strong membranous staining and other with weak diffused signal, reiterating the heterogeneity of PD-L1 expression among tumor cells (blue arrows)



- Variable PD-L1 expression level observed among tumor cells in clinical samples



## Materials and Methods



## Conclusion

Liquid biopsy provides accessible tumor information for disease monitoring and potentially therapeutic options. Here, we present results illustrating the successful integration of Clearbridge BioMedics' ClearCell® FX and Leica Biosystems' BOND RX for PD-L1 staining on clinical samples. This workflow can be used for study or further development of PD-L1 detection in liquid biopsy samples.

## References

- Hou, Han Wei et al. "Isolation and Retrieval of Circulating Tumor Cells Using Centrifugal Forces." *Scientific Reports* 3 (2013)
- <http://www.leicabiosystems.com/ihc-ish-fish/ihc-ish-instruments/products/leica-bond-rx/>