**Background** Colorectal cancer (CRC) is the third most diagnosed cancer in the United States with a projected 52,980 deaths in 2021. Microsatellite instability-high (MSI-H) CRCs with deficiencies in mismatch repair (MMR) are significantly associated with positive response to immunotherapy and improved outcomes when treated with immune checkpoint inhibitors. Programmed cell death ligand-1 (PD-L1) is an effective biomarker of MSI-H status to identify CRC patients who will respond to treatment, however, reproducible quantification of programmed cell death receptor-1 (PD-1)/PD-L1 in the tumor microenvironment (TME) across laboratory sites has been under-reported. In this study, our group directly addressed this issue by interrogating PD-1/PD-L1 cross-site at Akoya Biosciences and NeoGenomics Laboratories by employing the MOTiF™ PD-1/PD-L1 Panel kit along with the Vectra Polaris imaging system.

**Methods** Serial sections from 40 CRC samples with known MSI status were stained at Akoya and NeoGenomics Laboratories using a modified MOTiF PD-1/PD-L1 Lung Panel Kit on the Leica BOND RX. Sections were scanned using the Vectra Polaris imaging system at both sites. Inter-site staining reproducibility was assessed using image analysis algorithms developed with inForm tissue analysis software. Cell counts and densities were calculated using the R-script package Phenoptr-Reports and correlations were plotted per marker.

**Results** The average signal intensity for all markers/Opal fluorophores was within the recommended ranges of 10–30 normalized counts, with the exception of Polaris 780, which has an advised range of 1–10. This indicates the protocol stained successfully and reproducibly across all serial sections at both sites. Inter-site concordance analysis of cell densities for each marker yielded an average R2 value of ≥0.70. H-Score of PD-L1 quantified at the cell membrane trended with MSI status (stable/high).

**Conclusions** This study demonstrated that the MOTiF PD-1/PD-L1 Panel kit imaged in conjunction with the Vectra Polaris is not only a reliable assay that can be run across different sites, based on the concordant cross-site data, but that re-optimization of the kit allows for the assay panel to be successfully adapted to other cancers, such as CRC, which can then capture biological differences across a multitude of samples.

**REFERENCES**


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