Multiplex immunohistochemistry profiling with UltraPlex IHC on FFPE lung cancer provides a fast and robust staining platform compatible with pathology laboratory workflows

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ABSTRACT: A wide range of technologies are now available to perform multiplex immunofluorescence (mxIF) to examine the tumor immune microenvironment in FFPE tissue sections, offering the potential to reveal signatures of anti-tumor immune response. However, mxIF assay performance is often limited by autofluorescence and other artifacts, particularly in lung and prostate cancer samples. These artifacts can frustrate detection even using dedicated instrumentation to obtain images and advanced software to identify and measure the different biomarkers. Compared to immunofluorescence, immunohistochemistry (IHC) displays far less background and uses tools readily available in any pathology lab, but IHC has been traditionally difficult to multiplex especially regarding co-localization on cells. Recently, Cell IDx has overcome this barrier by developing UltraPlex™ multiplex IHC (mxIHC), where we leverage Cell IDx's antibody hapten-tagging technology to enable independent detection of 3-4 primary antibodies from any species on a single tissue section, using different chromogen colors to distinguish each biomarker. In this study of various NSCLC tumors, we display how mxIHC can define the TME while maintaining staining integrity. Using UltraPlex™ mxIHC, we demonstrate two useful chromogenic triple stains of CD8 / PD-L1 / panCK and CD163 / PD-L1 / CD68 with the ability to produce accurate quantitation for phenotype subsets. Our result show that UltraPlex™ mxIHC on FFPE tissue sections can provide increased confidence for multiplexing on challenging tissues while requiring only conventional laboratory methods and apparatus.

UltraPlex mxIHC Triplex: Continuous Flow with Three Steps

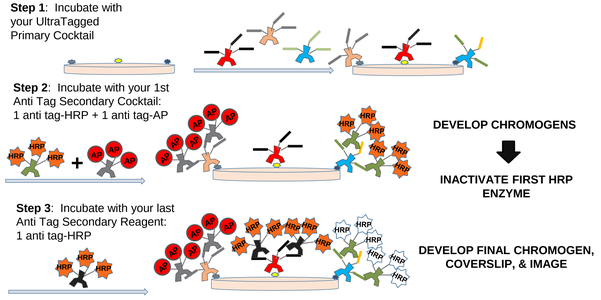

Figure 1: Schematic showing staining of tissue sections using modified hapten-labeled primary antibodies and detection using enzyme-labeled anti-hapten secondaries.

METHODS: Anti-human primary antibodies were conjugated to haptens (performed at Cell IDx). Antigen retrieval was performed on the Leica BOND RX using the ER2 protocol for 20 minutes. A protein block step consisting of 3% normal rabbit serum was applied for 20 minutes followed by an endogenous peroxidase blocking step before primary cocktail incubation. mxIHC primary anti human tagged panels were assembled into cocktails at a pre-determined concentration for each primary antibody. The primary cocktails were incubated for 30 minutes. After a washing step, the initial secondary anti-tag enzyme conjugated cocktail was prepared and added to slides for 30 mins. After incubation, slides were washed, and chromogens sequentially developed. Next, slides were incubated with HRP arrest buffer at 60C for 5 minutes, washed, and final anti-tag-HRP was added for 30-minute incubation. Final chromogen was developed, and slides were cover slipped using an aqueous mount. Slides imaged on the Aperio Versa 8 (Leica, Buffalo Grove, IL). Algorithms derived using Visiopharm with author capabilities software (Westminster, CO).

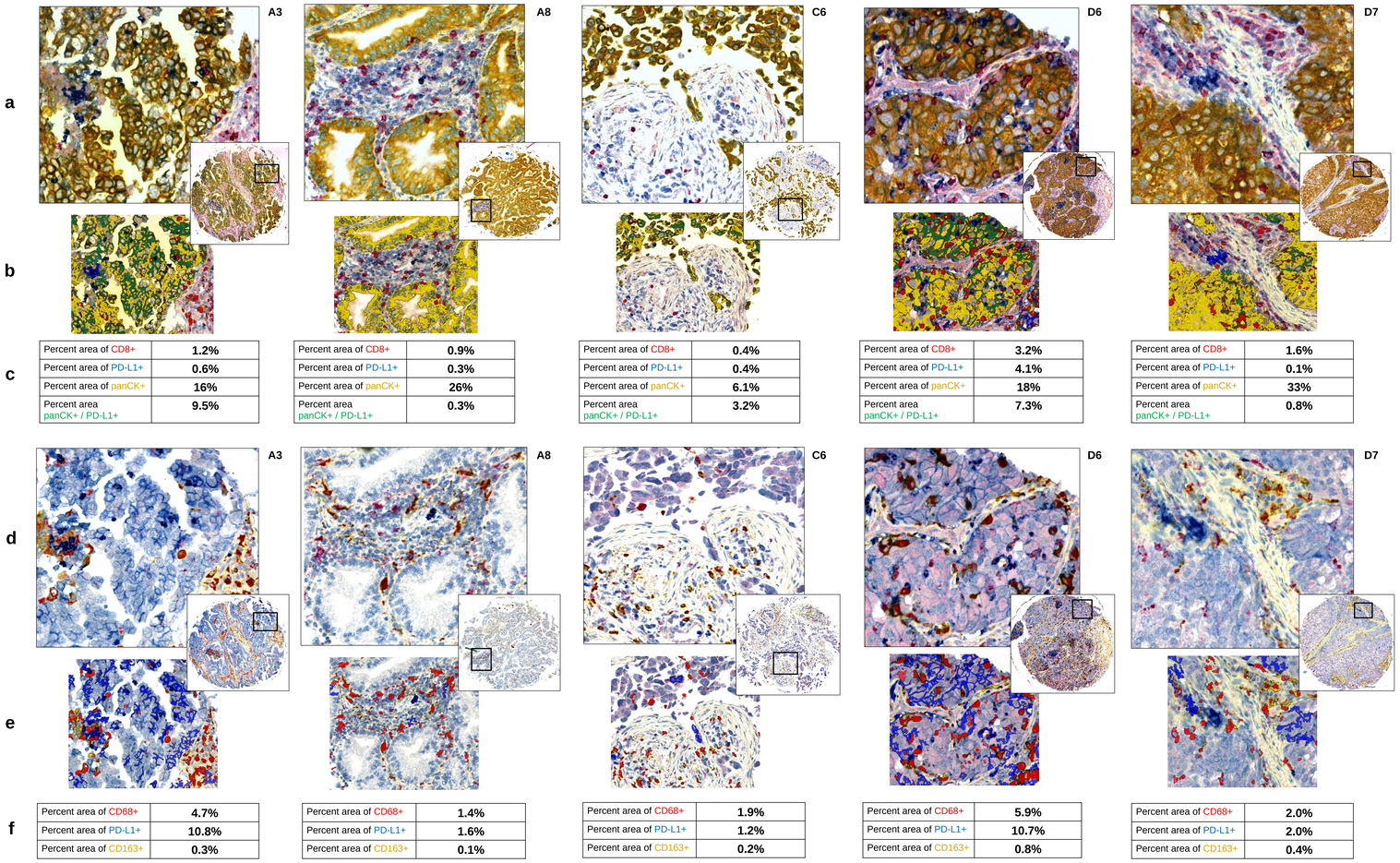

Figure 2 : **a** Cores imaged with the CD8 / PD-L1 / panCK mxIHC UltraPlex panel (20X magnification with whole core inset). **b** Virtual map images derived from the mxIHC images in "row a" using Visiopharm software with a single area pixel algorithm. **c** Percent area of each epitope from CD8 / PD-L1 / panCK panel calculated from the pixelated area of each chromogen color divided by the total area of the tissue core. **d** Cores imaged with the CD163 / PD-L1 / CD68 mxIHC UltraPlex panel (20X magnification with whole core inset). **e** Virtual map images derived from the mxIHC images in "row c" using Visiopharm software with a single area pixel algorithm. **f** Percent area of each epitope from CD68 / PD-L1 / CD163 panel calculated from the pixelated area of each chromogen color divided by the total area of the tissue core.

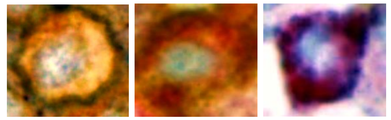

Figure 3: High magnification of double positive cells from chromogenic multiplex.

Advantages of UltraPlex mxIHC

- Primary antibody species independent
- Standard 4.5 hour, three-step staining protocol employed
- High throughput compatible
- Standard brightfield imaging equipment
- Data readily transferrable to image analysis software
- Conserved algorithm construction
- One HIER step with no stripping or harsh reagents to compromise your tissue
- Modular assembly compatible

CONCLUSION: Multiplex chromogenic assays have the ability to overcome low signal to noise ratios in problematic FFPE tissue specimens such as lung adenocarcinomas by avoiding problems with auto-fluorescence and other artifacts observed with fluorescent immunohistochemistry assays. The UltraPlex™ chromogenic immunohistochemistry assays employs one antigen retrieval step and was accomplished on the Leica BOND RX in ~4.5 hours without the limitations of primary antibody species compatibility and complex imaging equipment. In addition, area quantitative analysis obtained contextual pathological data using one algorithm per panel. Phenotype analysis for both panels displayed multiple phenotypes including PD-L1+/panCK+, CD8+, panCK+, CD68+, CD163+ cells and others. Pathological ratios between immune infiltrate and cancer cells may provide valuable insight and enable stratification in the tumor microenvironment. Both visual contrast and quantitation were accomplished in this experiment supporting reasons this technology could be implemented and adopted quickly with minimal disruption to workflow in a pathology laboratory setting.

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