A STRATEGY TO QUANTITATIVELY ASSESS THE ACCURACY AND PRECISION OF MULTIPLEX IMMUNOFLUORESCENCE ASSAYS – APPLICATION TO ULTIVUE INSITUPLEX® PD-L1, T-ACT AND APC PANELS

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Background Multiplex immunofluorescence assays represent an essential tool in immuno-oncology research. The recent past has witnessed the introduction of several multiplexing methodologies to detect multiple biomarkers on a single tissue section. The quantitative assessment of the accuracy and precision of multiplex panels is of paramount importance for their widespread use in clinical samples. While there have been numerous reports providing qualitative characterization of multiplex panels, there is a paucity of data concerning the quantitative validation of these panels.

Methods Ultivue Insituplex® T-act, PD-L1 and APC panels, which are 4-plex assays, were evaluated using breast tumor resections with varying levels of T-cell infiltration. For each panel, accuracy and precision was evaluated through a concordance and a reproducibility test, respectively. In the concordance test, five serial sections from each tumor specimen were cut and the 3rd (middle) serial section was immunolabeled with the 4-plex assay whereas the remaining sections were immunolabeled for the individual biomarkers (1-plex) that comprised the 4-plex assay. In the reproducibility test, five serial sections from each tumor specimen were immunolabeled with the 4-plex assay in separate, independent runs. The coefficient of variation (CV) of the density of different cell phenotypes was quantified from the serial sections and was used to assess the precision of that multiplex panel. All whole-slide image analysis was performed in QuPath software (version 0.2.3).

Results The results of the concordance test revealed that the relative difference in the single-biomarker cell density between 1-plex and 4-plex assays for the biomarkers in the 3 panels was typically less than 25%. Results of the precision test revealed that the CV for most cell phenotypes was typically less than 30%. We also identified special phenotypes such as CD3+PanCK+ cells and CD3+CD68+ cells, which exhibited unexpected combinations of biomarkers. Additional analysis revealed that these special phenotypes were in fact pairs of touching cells that were positive for the corresponding individual biomarkers (e.g., CD3+ cell touching a PanCK+ cell), and that this was due to limitations in the image analysis software package to segment the touching nuclei as two separate entities.

Conclusions Our results demonstrated that the Ultivue panels evaluated here had satisfactory accuracy and precision in breast tumor resections. The identification of special cell phenotypes in our data revealed the potential shortcomings of image analysis software and underscored the importance of performing a comprehensive evaluation of the multiplex assay as well as the image analysis workflow.

Ethics Approval The biospecimens used in the study were anonymized specimens which were collected with written patient consent, processed and distributed in full ethical and regulatory compliance with the Sites from which they were collected. This includes independent ethical review, Institutional Review Board approval (where appropriate), and independent regulatory review.

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