P19 *

Does batch testing impact the quality of multiplex immunofluorescence staining in large epidemiological cohorts of formalin-fixed paraffin-embedded tissues?

Purpose of the study

To validate novel multiplex immunofluorescence (mIF) staining to HRP-DAB immunohistochemistry (IHC) and to compare the mIF images produced across multiple staining batches in order to identify sources of bias that could influence accurate biomarker quantification.

Methods

Two novel mIF biomarker panels were assessed (NIB18-0282); multiplex panel 1 (MP1) which detected CD3, CD4, CD8 CD20, Ki67 and synaptophysin and multiplex panel 2 (MP2) for CD3, CD68, CTLA-4, PD-L1, STING and synaptophysin. Staining for each mIF panel was conducted in batches of 15 slides. Each batch of whole-face sections was run with a multi-tissue TMA as a mIF staining control. Slides were scanned using either an Aperio AT2 or PhenoImager HT. QuPath software was used to analyse both IHC and mIF images.

Summary of results

Correlation matrices show moderate-strong positive correlations (Rs>0.60) for mIF vs HRP-DAB IHC biomarker quantification in MP1 and MP2, validating panel design for all biomarkers except CTLA-4. Review of the slides found non-specific CTLA-4 staining present in HRP-DAB IHC but not mIF thus contributing to poor correlation (Rs=-0.11). Differences in cell mean intensities produced across control TMAs by staining batch in MP1 and MP2 was found to be acceptable (<2 Gy). However, evidence of dye-specific batch bias was observed in both MP1 and MP2 for Opal 520 antibody-opal pairings.

Conclusions

Staining using mIF sufficiently replicated biomarker analysis using HRP-DAB IHC validating MP1 and MP2 for the purposes of digital quantification. Use of mIF to target CTLA-4 produced cleaner reaction product for digital image analysis of the biomarker highlighting a strength of non HRP-DAB based detection systems. Staining batch effects were minimal across both panels; however, this study provides novel evidence that dye-specific batch effects can occur and may affect downstream image processing and biomarker quantitation.

A JSPS summer studentship supported this work.

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