



**QUEEN'S
UNIVERSITY
BELFAST**

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

James, H., Loughrey, P. B., McCombe, K., Salto-Tellez, M., & Craig, S. (2023). *Does batch testing impact the quality of multiplex immunofluorescence staining in large epidemiological cohorts of formalin-fixed paraffin embedded tissues?*. Poster session presented at 5th Joint Winter Meeting of the Pathological Society and the Royal Society of Medicine 2023: unleashing the edge power of pathology , London, United Kingdom.

Document Version:

Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:

[Link to publication record in Queen's University Belfast Research Portal](#)

General rights

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Open Access

This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: <http://go.qub.ac.uk/oa-feedback>

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 Phenolmager HT. QuPath software was used to analyse both IHC and mIF images.

Summary of results

Correlation matrices show moderate-strong positive correlations ($R_s > 0.60$) 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 ($R_s = -0.11$). 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.

@James, H.; Loughrey, P.B.; McCombe, K.D.; Salto-Tellez, M.; Craig, S.G.
Queens University Belfast, Belfast, United Kingdom ;