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Title: MultiOmyx: A Novel Multiplex Methology for the Evaluation of Hodgkin Lymphoma

Background/Introduction: The diagnosis of classical Hodgkin lymphoma is often difficult to establish due to the rarity of the neoplastic component and the necessity to perform immunostains on serial sections. We have developed a fluorescent multiplexed assessment of CD30-positive cells with eight additional antibodies in formalin-fixed, paraffin-embedded sections (MultiOmyx) which enables assessment of multiple antigens on a single tissue section and allows evaluation of specific cells within specific fields.

Methods: Directly conjugated fluorescent antibodies were applied to a slide, followed by whole slide imaging. The dye was chemically inactivated, enabling a second round of staining with another fluorescent antibody. This process was performed multiple times on that single slide. The gray scale fluorescent images were transformed into virtual bright-field images which closely resembled conventional images, and also enabled direct comparison of antibodies within the same field. The antibodies used included CD30, CD15, PAX-5, CD45, CD20, CD79a, OCT-2, BOB.1, and CD3. The stains were analyzed for the cellular and subcellular staining distribution and whether any non-specific staining was present.

Results: Forty samples were analyzed, including 34 unique specimens, of which 17 represented classical Hodgkin lymphoma and the rest included both reactive and lymphoma tissues in the differential diagnosis with Hodgkin lymphoma. Appropriate cellular and subcellular localization was seen with the fluorescent MultiOmyx technology for all antibodies, with direct comparison of the results of one antibody with another. There were 5 process failures out of 360 imaging rounds for MultiOmyx vs. 2 out of 333 for immunohistochemistry. Concordance in specificity between the fluorescent images and their corresponding brightfield immunohistochemical stains was found in >97% of cases. Although not quantified for this assessment, the fluorescent staining intensities generally were similar to the immunohistochemical stains.

Discussion: This novel MultiOmyx technology has similar staining characteristics as standard immunohistochemical stains, and has the added advantage that it may be performed on a single section. This allows for better correlation of results between stains in a given case, particularly in cases with rare Hodgkin cells, since the technology allows direct comparison of stains within the same field of view. The MultiOmyx technology may be performed in small samples, in which full immunohistochemical profiles are not possible to obtain.