Demonstration of Rapid Immunocytochemistry
Resolve Molecular Targets in 10-20 Minutes

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BACKGROUND

The Novodiax ihcDirect® family of IVD products are capable of 10-minute immunohistochemistry (IHC) staining on frozen tissue sections[1]. The technology is derived from a proprietary platform of polymerized horseradish peroxidase (polyHRP) directly conjugated to a primary antibody. Such rapid IHC assays enable the timely intra-operative evaluation of resection margins (e.g. Mohs micrographic surgery[2]) as well as intra-operative differential diagnosis (e.g. breast cancer surgery[3]). Consequently, there is now a great deal of interest in fast and sensitive biomarker-based assays that could similarly enable the rapid on-site evaluation (ROSE) of cytology specimens to help determine sample adequacy and triage biopsy material appropriately without having to wait for time-consuming cell block preparations. The objective of the present study is to begin to characterize novel applications of Novodiax's ihcDirect polyHRP-conjugated antibodies for rapid immunocytochemistry (ICC) staining on freshly prepared whole cell samples.

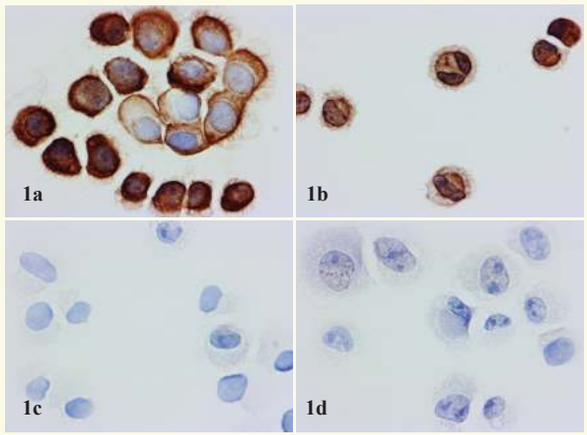

Figure 1. Application of Novodiax ihcDirect Pan-CK 4Abs on fresh cell samples. 1a: SK-BR-3 cell line (mammary gland/breast, derived from metastatic site); 1b: SiHa cell line (cervix, squamous cell carcinoma); 1c: SK-MEL cell line (skin, malignant melanoma); 1d: A375 cell line: (skin, malignant melanoma). 1a and 1b show positive staining, while 1c and 1d show negative results.

DESIGN

Novodiax polyHRP-conjugated AE1/AE3, CD20, CD45, CK5, CK 8/18, Ki67, Mart-1, Pan-CK 4Abs, and SOX10 antibodies were used to perform ICC testing on cell smears prepared from human cheek cells and cyto-centrifuged samples of cultured cell lines: SiHa (cervical cancer), A375 (melanoma, SOX10+, Mart-1-), SK-MEL (melanoma, SOX10+, Mart-1+), SK-BR3 (breast cancer), GA-10 (B lymphocyte), and MJ[G11] (T lymphocyte). Freshly prepared specimens were briefly air dried to ensure proper adherence to the microscope slides. Subsequently, specimens were pretreated with one of four fixatives in order to fix and permeabilize the cells. These fixatives included acetone, 10% neutral buffered formalin (NBF), alcohol (40% EtOH in PBS) and Dent (80% MeOH, 20% DMSO). Novodiax ihc Blocker was used in the staining procedure to reduce the occurrence of background staining, and 3,3'-diaminobenzidine (DAB) was used as the chromogenic substrate. Antibody and chromogen incubation times were varied according to the needs of the specific antibody and specimen type, ranging from 3 - 15 min. for the antibody, and 3 - 5 min. for DAB chromogen.

RESULTS

ICC staining was successfully completed within 10-20 minutes for each of the Novodiax polyHRP-conjugated antibodies on all of the cell preparations. Variability in stain quality was observed according to the type of fixative used. Staining results by fixative type are summarized in Table 1, where check marks represent satisfactory results. Examples of satisfactory positive and negative staining, using Novodiax ihcDirect Pan-CK 4Abs, are shown in Figure 1. Positive staining with ihcDirect CD20 and ihcDirect AE1/AE3 is shown in Figure 2. Some of the specimens displayed poor morphology as a result of sub-optimal quality of the cultured cell lines.

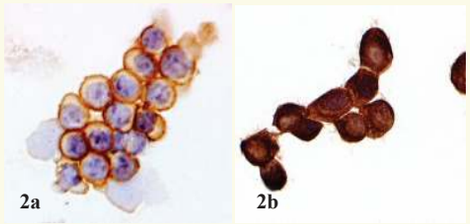

Figure 2. 2a: GA-10 cell line (B lymphocytes, stained with Novodiax ihcDirect CD20); 2b: SK-BR-3 cell line (breast cancer cell, stained with Novodiax ihcDirect AE1/AE3).

✔ *indicates satisfactory staining*

Table 1.	polyHRP conjugated antibodies								
	AE1/AE3	CD20	CD45	CK5	CK 8/18	Ki67	Mart-1	Pan-CK 4Abs	SOX10
Acetone	✔	✔	✔	✔	✔	✔	✔	✔	✔
10% NBF	✔	--	✔	--	✔	✔	✔	✔	✔
EtOH	✔	--	✔	--	--	--	--	--	--
Dent	--	✔	--	--	--	--	--	--	--

DISCUSSION

In the present study it is demonstrated that, similar to 10-minute IHC on frozen sections, Novodiax ihcDirect polyHRP-conjugated antibodies may be utilized for rapid ICC to obtain satisfactory results in as short as 10-20 minutes. This suggests a potential utility for rapid on-site evaluation (ROSE) to resolve biomarker targets at the molecular level without having to wait for embedding cytology specimens into cell blocks. The results of this study also demonstrate the critical role that specimen pretreatment plays in the quality of staining—specifically fixative type. For the antibodies and specimen types that we have tested, acetone and 10% NBF appear to be the optimal fixatives. Further study of the impact of different fixatives and fixation times is required in the future. Additional studies are also needed for assessing other available Novodiax polyHRP-conjugated antibodies beyond those included in this study, and determination of optimal working conditions for other widely used fresh clinical cytology specimens (e.g. fine needle aspirates, core needle biopsies, blood smears, touch imprints, etc.)

[1] Liu, Mei et al. A Direct Immunohistochemistry (IHC) Method Improves the Intra-operative Diagnosis of Breast Papillary Lesions Including Breast Cancer. *Discov Med* 28(151):29-37, July 2019.

[2] Sroa N, Campbell S, Ravitskiy L. Immunohistochemistry in mohs micrographic surgery: a review of the literature. *J Clin Aesthet Dermatol.* 2009;2(7):37–42.

[3] Liu, Haiyan. Application of immunohistochemistry in breast pathology a review and update. *Arch Pathol Lab Med.* Vol 138:1629-1642. Dec 2014.