Rapid and sensitive multiplexing approach with Novodiax ihcDirect™ Technology

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Background

Multiplex testing can optimize the use of precious specimens, provide better insights into the local microenvironment, reveal a better understanding of co-expression and spatial organization of biomarkers and/or distinguish different cells in blood samples. The Novodiax ihcDirect™ technology, in comparison to conventional immunohistochemistry (IHC), provides a simpler and faster test procedure. Novodiax uses a novel polyHRP-antibody detection system to improve both intraoperative IHC on frozen tissues as well as immunocytochemistry (ICC) on fresh cells. Tests for both processes can be completed in just 10 minutes while demonstrating superior detection sensitivity and maintaining low background across a wide variety of antibodies. Thus, the multiplex approach with Novodiax ihcDirect technology can be a powerful tool to investigate for hidden clues in cell and tissue samples in a rapid fashion. For instance, a triplex IHC/ICC on fresh tissue or cells can be finished within an hour. Recently, this technology has been applied to the analysis of blood samples in liquid biopsy for detecting circulating tumor cells using both colorimetric and fluorescent substrates.

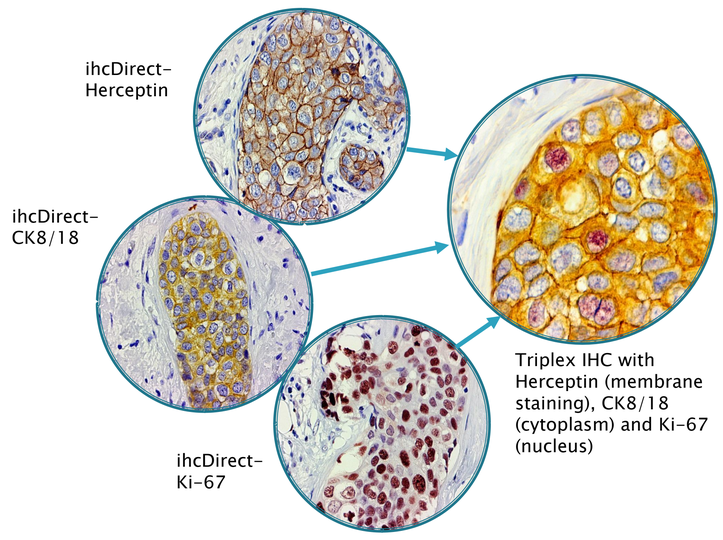

Figure 1. Demonstrates a colorimetric IHC multiplex on Breast Cancer formalin-fixed paraffin-embedded (FFPE) tissue. Tissues were stained using ihcDirect Herceptin, ihcDirect CK8/18, and ihcDirect Ki-67. The cancer cell membrane was highlighted with brown color, the cytoplasma with bright yellow, and the nucleus with dark red.

Results & Discussion

Table 1. A comparison between Novodiax IHC and conventional IHC

	Novodiax IHC	Regular IHC
Parallel multiplex IHC		
Species limit for primary Ab	No	Yes
Speed	Fast	Slow
Sequential multiplex IHC		
Stripping off primary Ab	No	Yes
Diminished signal	No	Yes
Blocking between IHC	No	Yes
Speed	Fast	Slow

Procedure	Novodiax IHC on FF	Novodiax IHC on FFPE	Other IHC on FFPE
Dewax + AR		60	60
Block	1	30	30
1st IHC	9	45	105
Stripping or HRP deactivation	10	10	30
Block			30
2nd IHC	9	45	105
Stripping or HRP deactivation	10	10	30
Block			30
3rd IHC	9	45	105

Sequential multiplexing of IHC using the Novodiax ihcDirect technology uses a simple 1-step deactivation procedure that requires no antibody stripping. Per Table 1, this method provides a fast and easy way for users to observe multiple bio-markers on frozen or FFPE tissue sections. Thus, this approach is very convenient for researchers to obtain more information from a single sample.

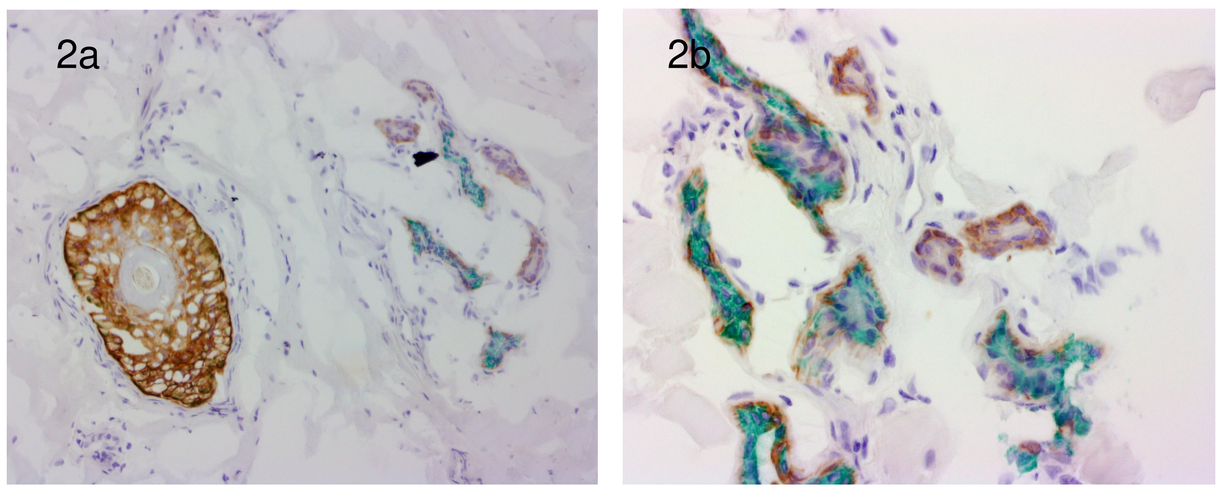

Figure 2. Application of a duplex IHC on a Frozen Skin Tissue. The tissue was stained with ihcDirect CK5 (Brown) and ihcDirect CK8/18(Green).

To more accurately determine margins in surgical treatment of skin cancers, we applied ihcDirect CK5 onto intra-operative frozen sections. CK5 is a cytokeratin that has demonstrated utility when directed at basal cell carcinoma and squamous cell carcinoma and on surgical margins during the skin cancer surgery. However, often the morphology of exocrine gland cells, such as sweat glands, or sebaceous glands can make it difficult to distinguish gland tissue from cancer cells. This situation can make difficult for doctors to provide an accurate diagnosis. As shown in Figure 2, the use of a duplex stain, ihcDirect CK8/18 highlighted the sweat gland. Notably, the overall procedure of using this duplex IHC staining was approximately 30 minutes.

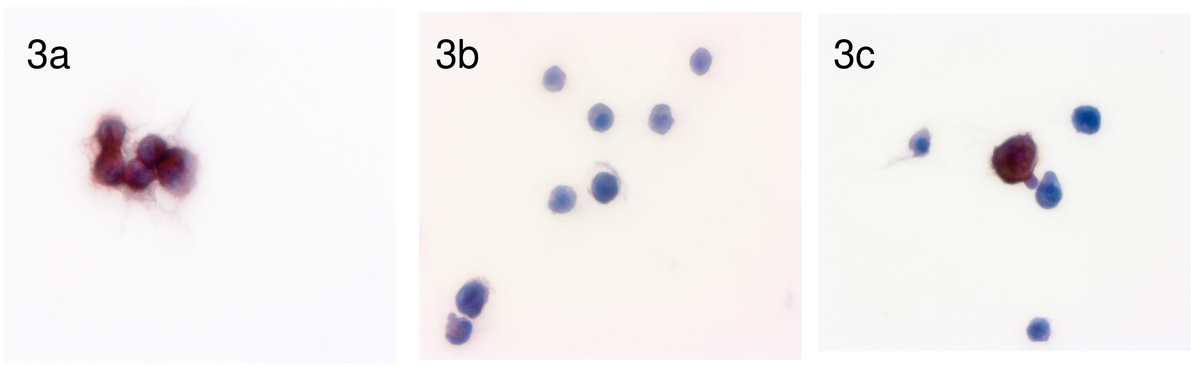

Figure 3. Application of Novodiax ihcDirect technology on a fresh cell samples. 3a: Breast Caner cell stained positive by ihcDirect-Herceptin; 3b:Melanoma cell stained negative by ihcDirect-Herceptin; 3c: Melanoma cell sample spiked with Breast Cancer cell, the breast cancer cell was selectively stained by ihcDirect-Herceptin. All ihcDirect-antibodies were developed by Novodiax Inc. The fresh breast cancer cell was SK-Br3, and the melanoma cell was SK-Mel. Before the ICC staining ,the cell culture was added onto a clean slide and dried in a 37ºC incubator, then fixed for 30 seconds in a formalin fixative.

A similar strategy can also be applied to fresh cell samples obtained from liquid biopsies. Under these conditions the Novodiax ihcDirect polyHRP technology permitted rapid visualization of different cells specific to the conjugated antibody.

Conclusion

Rapid multiplex testing of specimens using Novodiax ihcDirect for IHC/ICC is achievable and shows great promise to aid researchers and medical professionals obtain timely and useful information. This technology facilitates fast and accurate diagnosis for frozen tissues, FFPE and circulating tumor cells applied to basic research and medicine.