Rapid Immunohistochemistry, Multiplex IF and Digital Pathology Workflow

Authors Jennifer Freeland, MS, HTL(ASCP)cm and Nathan Schwandt, BSHS, HTL (ASCP)

epredia Enhancing precision cancer diagnostics

Overview

Rapid immunohistochemistry using Fast Fluidic Technology (FFeX) enables precise and efficient advanced staining techniques, including monoplex and multiplex immunohistochemistry (IHC) and immunofluorescence (IF). Scanning by 3DHistech™ digital pathology solutions enables pathologists to evaluate and collaborate rapidly remotely and with transparency and consistency, thus improving the pathology workflow. Together, the LabSat® Research and Pannoramic Midi II demonstrate a rapid IHC and digital scanning solution.

Introduction

Methods for IHC, IF, and multiplexing that deliver high quality stain results and maintain tissue morphology are important for effective tissue research. For example, IHC and IF have been identified as reliable methods that contribute to the study of the tumor microenvironment (TME) (Taube, et al 2020). In addition, technologies that offer high staining quality and morphology while improving workflow efficiencies may further enable the research of important biomarkers. This study highlights rapid IHC and multiplexing IF coupled with efficient and high quality scanning through 3DHistech systems.

Methods

Sample preparation

TMAs: Formalin fixed paraffin embedded (FFPE) tissue microarray slides were purchased from a commercial resource (Pantomics). Four micron sections of TMA tissues (breast cancers, known positive controls for estrogen receptor and progesterone receptor) were deparaffinized and rehydrated to deionized water following laboratory procedures. Tonsil sections: 4 micron thick sections were made from formalin fixed paraffin embedded tonsil tissues. The FFPE blocks were purchased from a commercial resource (BioIVT). Sections were allowed to air dry at ambient temperatures overnight and were deparaffinized and rehydrated to deionized water following laboratory procedures.

Figure 1: The entire IHC workflow using the Lunaphore LabSat® Research and 3DHistech Pannoramic Midi II requires just under an hour (58 minutes).

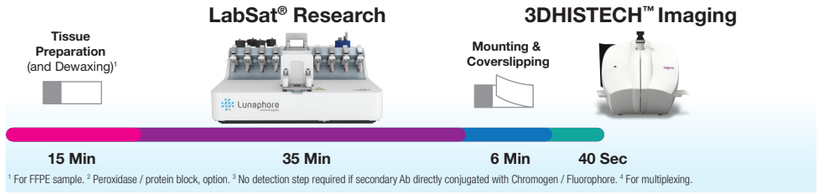
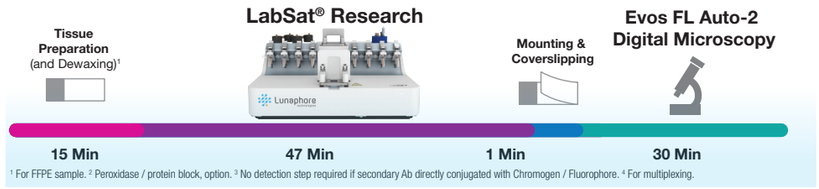


Figure 2: The entire multiplex IF workflow using the Lunaphore LabSat® Research and Evos FL-Auto requires just over an hour and a half (93 minutes).



IHC staining

Heat induced epitope retrieval (HIER) of the TMA and tonsil sections was performed on the LabSat instrument using Epredia HIER Buffer L (pH 6) TA-135-HBL or HIER Buffer M (pH 8) TA-135-HBM. Antibodies used in the study included Ki67 (RM-9106-S) diluted 1:100 with Primary Antibody Diluent (TA-125-ADQ), Her2 (RM-9103-S) diluted 1:100, by Epredia. The IHC detection kit system, UltraVision Quanto Detection System HRP / DAB (TA-125-QHD) was placed on the Lunaphore LabSat® instrument. Counterstain included Modified Mayer's Hematoxylin (TA-125-MH). The IHC staining protocol on LabSat is located in Table 1, and requires a total of 34 minutes and 27 seconds for completion. The slides were removed from the Lunaphore LabSat® instrument, dehydrated through graded alcohols, mounted with a permanent mounting medium (Epredia Mounting Medium 4111) and allowed to air dry.

Multiplex IF staining

Heat induced epitope retrieval (HIER) of the TMA sections was performed on the LabSat® instrument using Epredia HIER Buffer L (pH 6) TA-135-HBL. The Cell IDx UltraPlex mxIF BC-1A Panel was utilized as the immunofluorescent cocktail multiplex stain. The antibodies in the panel are labeled with 490, 550, 650, and 750 nm fluorophores (Cell IDx 2020). The antibodies in the cocktail include estrogen receptor (ER), progesterone receptor (PR), Ki67, and Her2. The TMA sections were known positive controls for ER and PR, but negative for Her2. The multiplex IF (mIF) staining protocol on LabSat® is located in Table 2, and required a total of 47 minutes and 11 seconds for completion. The slides were removed from the Lunaphore LabSat® instrument, mounted with an aqueous mounting medium (PermaFluor, TA-006-FM) and allowed to air dry.

Table 1: IHC staining protocol on the Lunaphore LabSat® for Ki67 and Her2

Lunaphore LabSat Protocol (IHC)		
Step	**Reagent**	**Time (min)**
1	HIER Buffer L pH 6	5
2	Quanto H2O2 Block	0.5
3	Quanto UV Block (Protein Block)	0.5
4	Epredia Ki67 SP6 or Her2 SP3 1:100	4
5	Quanto Amplifier	2
6	Quanto HRP Polymer	2
7	Quanto DAB	0.25
8	Modified Mayer's Hematoxylin	0.25

Table 2: mIF staining protocol on the Lunaphore LabSat® for cocktail antibodies ER, PR, Ki67, Her2

Lunaphore LabSat Protocol (mIF cocktail)		
Step	**Reagent**	**Time (min)**
1	HIER Buffer M pH 6	10
2	Cell IDx Blocking Solution	2
3	Primary Antibody Cocktail (all 1:100)	8
4	Secondary Antibody - Fluorophore Cocktail	8
5	Final rinse	0.25

Figure 3: Tissue microarray of Her2 positive breast carcinoma (3+); IHC stain for Her2 (RM-9103-S, 1:100) and Quanto HRP/DAB detection. Image taken with the 3DHistech Pannoramic Midi II (20x).

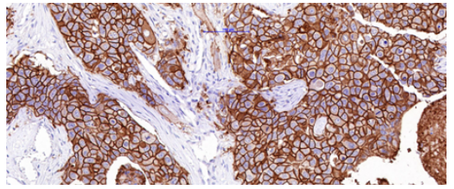


Figure 4: Tonsil; IHC stain for Ki67 (RM-9106-S, 1:100) and Quanto HRP/DAB detection. Image taken with the 3DHistech Pannoramic Midi II (20X).

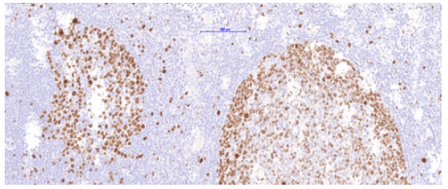


Figure 5: Tissue microarray of ER and PR positive (Her2 negative) breast carcinoma; multiplex IF stain for ER, PR, Ki67, Cell IDx. Image taken with EVOS FL-Auto 2 (20x).

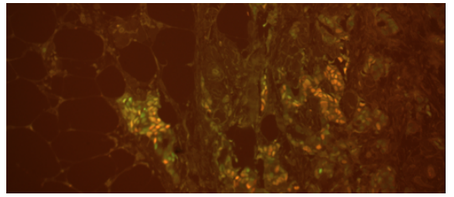
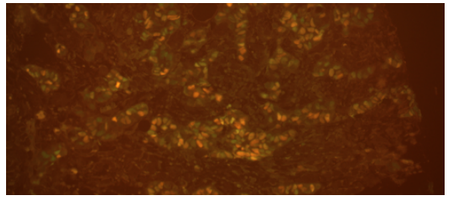


Figure 6: Tissue microarray of ER and PR positive (Her2 negative) breast carcinoma; multiplex IF stain for ER, PR, Ki67, Cell IDx. Image taken with EVOS FL-Auto 2 (20X).



Digital Pathology

Dried, coverslipped IHC slides were scanned with the Pannoramic Midi II using an IHC profile. The slides were loaded into the slide tray and scanned in approximately 40 seconds each. Scanned slides were examined and areas of interested were selected for photomicrographs. Digital images of Her2 IHC and Ki67 IHC can be observed in Figures 3 and 4.

Multiplex IF slides were scanned with the EVOS FL Auto 2 digital microscope. The cubes in the system included those for GFP, RFP, Qdot 655, and DAPI. Each slide took approximately 30 minutes to examine, select areas of interest, and collect digital micrographs. Digital images of ER (green), PR (yellow) and Ki67 (red) can be observed in Figures 5 and 6.

Conclusion

Efficient workflow for IHC and multiplex IF staining and digital pathology scanning is illustrated using the Lunaphore LabSat® and the 3DHistech Pannoramic Midi II and the Evos FL Auto-2. The workflow rapidly delivered high quality, precise results. Most current automated and manual IHC and IF options require approximately 2.5 to 3.5 hours to complete a stain. By adding in the dehydration, coverslipping, and digital microscopy steps, the total required time could come to between 3 and 4 hours from the FFPE slide to the digital image. A more on-demand workflow was realized by the implementation of the Lunaphore LabSat® Research and the 3DHistech Pannoramic Midi II. Using this workflow, the total IHC staining and imaging time was reduced by 66 to 75%, and multiplex IF staining and imaging was reduced by 50 to 60%.

References

- Brajkovic A, Pelz B, Procopio MG, et al. (2018). Microfluidics-based immunofluorescence for fast staining of ALK in lung adenocarcinoma. *Diagnostic Pathology.* 13:79. Doi:10.1186/s13000-018-0757-1
- Cappi G, Dupoy DG, Comino MA, et al. (2019). Ultra-fast and automated immunohistofluorescent multistaining using a microfluidic tissue processor. *Scientific Reports.* 9:4489. Doi: 10.1038/s441598-019-41119-y
- Cell IDx. (2020). UltraPlex mxIF: Multiplex immunofluorescence staining protocol for panels supplied with indivisual reagents. www.cellidx.com
- Ciftlik AT, Lehr HA, and Martin AM. (2013). Microfluidic processor allows rapid HER2 immunohistochemistry of breast carcinomas and significantly reduces ambiguous 2+ read-outs. *PNAS.* 110(14): 5363-5368
- Epredia. (2016). Data Sheet: c-erB-2/Her2/neu (SP3). www.epredia.com
- Epredia. (2016). Data Sheet: Estrogen receptor (SP1). www.epredia.com
- Epredia. (2016). Data Sheet: Progesterone receptor (SP2). www.epredia.com
- Epredia. (2016). Data Sheet: Quanto Detection System HRP / DAB (SP1). www.epredia.com
- Taube JM, Akturk G, Angelo M, et al. (2020). The Society for Immunohistochemistry of Cancer statement on best practices for multiplex immunohistochemistry (IHC) and immunofluorescence (IF) staining and validation. *Journal for ImmunoTherapy of Cancer.* 8:e000155. Doi:10.1136/jitc-2019-000155