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Advancing immunoassays through innovation

Development of a practical 10-min intraoperative IHC product line

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Introduction

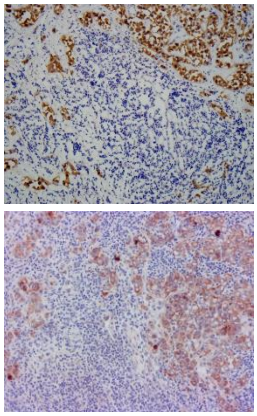
Intraoperative frozen section (FS) diagnoses are expected to be completed within 20 minutes (min). The conventional immunohistochemistry (IHC) has a very limited application because it requires at least 2-3 hours finishing the staining process. Numerous attempts have been made to develop a fast IHC on FS. However, all previous attempts have failed because these procedures were too complicated, costly, or unable to be applied to all important diagnostic markers. Using a unique polymerized horseradish peroxidase (pHRP), we successfully developed a 10-min IHC assay for the most commonly used antibodies on FS at room temperature.

Materials & Methods

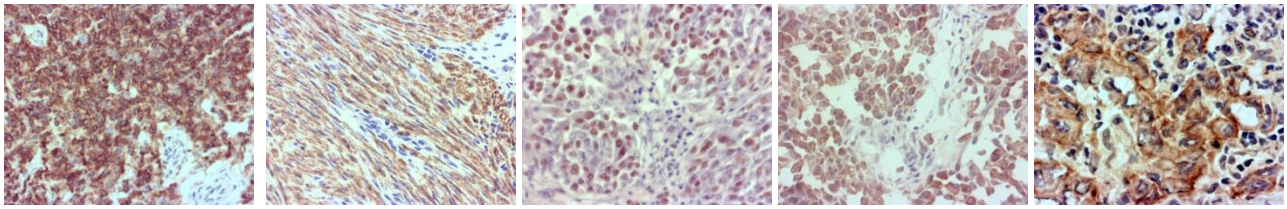
A unique PolyHRP optimized based on shape, size, and enzyme activity was covalently conjugated to 10 primary antibodies against the popular biomarkers in the field of anatomic pathology: pan-cytokeratin, cytokeratin 8/18, Mart-1, SOX-10, synaptophysin, desmin, smooth muscle myosin, CD45, GFAP and Her2. The antibodies were either licensed from a university (such as, pan-cytokeratin, clones AE1/AE3) and Mart-1, clone A103) or developed in house or purchased from a pharmacy (such as, human antibody against Her2, Herceptin). All conjugates were first validated intensively on FFTE tissues and then tested on frozen tissues.

Results

We have developed a single protocol for different pHRP-conjugated antibodies by adjusting the concentration, incubation time, and blocking time (see the table below). This protocol worked well at room temperature between 22°C and 25°C. Under lower temperature (e.g., <20°C), the weak results have been observed. A device that can maintain 30°C has been used to overcome this problem and to provide additional standardization. Representative cases on frozen tissues are shown below.



Procedure	Time in min
Wash after fixation	0.5
Blocking	1
PolyHRP-Antibody	3
Wash	0.5
DAB	3
Wash	0.5
Counter Staining + Aqueous Media Mounting	1
Total	**10**



Demonstration of 10-min IHC results for pHRP-antibodies on frozen tissues: (1) pan-cytokeratin on a lymph node section with invasive breast cancer cells, (2) CK 8/18 on a lymph node section with invasive breast cancer cells, (3) CD45 on Hodgkins lymphoma, (4) SMMS-1 on uterus, (5) SOX-10 on melanoma, (6) Mart-1 on melanoma, (7) pHRP-Herceptin on a frozen lymph node section with invasive breast cancer cells. Results in Figures 1-6 were obtained under room temperature, while pHRP-Herceptin result was obtained using a 30°C device.

Conclusions

We have developed a robust HRP polymerization technology, which can directly modify primary antibodies and omit the use of secondary antibodies on a rapid IHC with sufficient detection sensitivity. Using pHRP conjugated antibodies with a 10-min IHC staining protocol will allow pathologists to evaluate surgical margins and invasive cancer cells in sentinel nodes on FS. We are continuously developing more 10-min IHC tests. Without secondary antibody, this technology allows us to convert therapeutic antibodies to diagnostic tools, providing more accurate and logical companion diagnostic message. Our pHRP-Herceptin works well on frozen tissue section with the 10-min protocol. Testing Her2 status on frozen tissue section may potentially reduce the possible false negative result caused through the harsh process of formalin-fixation, paraffin-embedding, dewaxing and antigen retrieval. A study using more than 100 archived breast cancer tissues has demonstrated superior accuracy of pHRP-Herceptin over several FDA approved Her2 tests in the market (A poster presentation in USCAP 2016. Available upon request).