Optimization of Rapid Immunohistochemical Stains on Frozen Tissue Sections

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BACKGROUND

Intraoperative frozen section consultation plays an important role in patient management. However, accurate diagnosis can sometimes be challenging. The application of rapid immunohistochemistry (IHC) on frozen sections, recently introduced by NovoDiax, Inc. (Hayward, CA), can be useful when working on a difficult case. The aim of this study is to test and optimize the staining conditions for this rapid IHC technique using horseradish peroxidase (HRP) polymer labeled mouse anti-human AE1/AE3 antibody.

MATERIALS AND METHODS

Fresh frozen sections of the colon (N=3) and fallopian tube (N=2) were used to evaluate and optimize the ihcDirectTM Anti Pan-Cytokeratin Kit (Clone AE1/AE3) under various conditions as summarized in Table 1. A total of 30 protocols/ combinations was generated from Table 1 and tested on the 3 colon and 2 fallopian tube samples. The optimal staining protocol, defined as the least performance time with appropriate staining signals and well-preserved histomorphology, was determined, illustrated in Figure 1a. The optimal protocol was validated on 40 real frozen section cases from variety of tissues/ tumors as summarized in Table 2. Representative images are demonstrated in Figures 1b to 1h.

Table 1. Summary of Staining Conditions

Fixative	Fixation Time	Incubation Time	Temperature
1. Cold acetone	1. 30 seconds	1. 3 minutes	1. Room (22^{0}C)
2. Frozen section	2. 1 minute	2. 5 minutes	2. 30^{0}C
fixative solution*	3. 2 minutes	3. 10 minutes	3. 37^{0}C

*25 ml of glacial acetic acid, 100 ml of 37% formaldehyde, and 375 ml of methyl alcohol.

Table 2. Application of Rapid IHC on 40 Real Frozen Section Cases

AE1/AE3 (30"-1'/30^{0}C/3')	N of Cases	N of Neg.	N. of Pos. (dist., Int.)	Background
Mets, LN	2	0	2 (4+, w-m)	Weak
Mets, Other	4	1	3 (3-4+, m)	Weak
Lung and Pleural	5	0	5 (3-4+, m)	None
Oral Cavity	4	0	4 (3+, w-m)	Weak
Uterus	10	0	10 (2-4+, m-s)	None
Ovary	3	0	3 (3-4+, m-s)	None
Salivary Gland	4	0	4 (4+, m)	None
Ureter	2	0	2 (4+, m)	Weak
Kidney	1	0	1 (3+, m)	Weak
Pancreas	1	0	1 (4+, m)	Weak
Skin	1	0	1 (4+, m)	Weak
Urinary Bladder	1	0	1 (4+, m-s)	None
Breast Tissue	2	0	2 (4+, m)	Weak

Mets - metastases; LN – lymph node; Neg. – negative; Pos. – positive; Dist. – distribution; Int. – intensity; N – number; w – weak; m – moderate; s – strong
1+ (5-25%); 2+ (25-50%); 3+ (50-75%); 4+ (>75%)

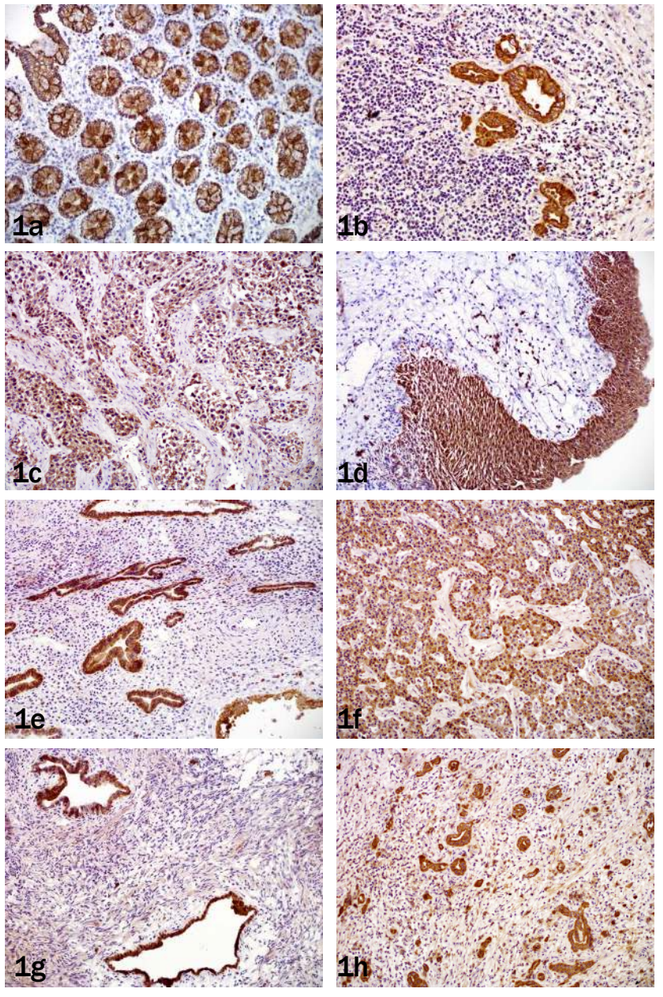

Figure1a. Using the optimal staining protocol (30"/3'/300C) on a test colon specimen, the epithelial cells show good immunostaining signal for AE1/AE3 with clean background.

Figures 1b to 1h. Validation of the optimal staining protocol on variety of real frozen section tissues/tumors. **1b**, metastatic adenocarcinoma to lymph node; **1c**, metastatic squamous cell carcinoma to soft tissue; **1d**, urothelial hyperplasia; **1e**, benign endometrial tissue; **1f**, pancreatic neuroendocrine tumor, grade 1; **1g**, endometriosis of the ovary; **1h**, metastatic adenocarcinoma to pleura.

RESULTS

1) Cold acetone was a better fixation than the frozen fixative solution; 2) there were no significant differences among the three fixation times; 3) prolonged antibody incubation time such as 10 minutes yielded slightly stronger signals and background staining; in contrast, 3-minute antibody incubation provided adequate staining signals with no background staining; 4) no staining or only weak staining was observed when the IHC assay was conducted at 22^0 with 3-minute antibody incubation; in contrast, much stronger staining signals can be obtained at 30^{0}C. When raising the temperature to 37^{0}C, tissue preservation was poor. When utilizing this technique on real frozen tissue sections, the fixation time can be increased to 1' in order to achieve optimal results, however, weak background staining was observed in 43% (17/40) of cases.

CONCLUSIONS

The application of IHC on frozen tissue sections is practical and can be completed within 10 minutes. The optimal staining protocol for ihcDirectTM Anti Pan-Cytokeratin Kit (Clone AE1/AE3) is 1) fixed frozen section in cold acetone for 30 seconds to 1 minute; 2) incubated with the antibody for 3 minutes; and 3) IHC assay performed at 30^{0}C. Further testing on various fresh frozen tissues with variety of antibodies is needed before utilizing this new IHC method for patient care.

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