Pitfalls in Immunocytochemical Study Using Fine Needle Aspiration Samples

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57.1 Introduction

Immunostaining of histopathological specimen is carried out using formalin-fixed paraffin-embedded tissues with or without antigen retrieval. Meanwhile, immunostaining of fine needle aspiration (FNA) smear is usually carried out using 95% alcohol-fixed specimens. The immunostaining results obtained with histopathological specimens and those with cytological specimens may be discrepant or even contradictory due to the differences in fixatives, fixation methods, and/or antigen activation treatment. An automated immunostaining instrument in our hospital is used for the special care in fixation of cytological smears. For FNA smear, alcohol fixation is used, which is followed by further fixation with phosphate-buffered formalin solution and an antigen activation treatment. Useful immunostaining markers in diagnosis of thyroid tumors are shown in Table 57.1.

57.2 Methods

57.2.1 Protocol for Immunohistochemistry Using Formalin-Fixed Paraffin-Embedded Specimens

Immunohistochemical study is performed with 4 μm serial sections prepared from formalin-fixed, paraffin-embedded tissue blocks. Immunostaining is carried out with an automated immunostainer, the Ventana BenchMark XT device (Ventana, Tucson, AZ), using a streptavidin-biotin-peroxidase kit with 3,3′-diaminobenzidine (LSAB, Ventana).

1. Deparaffinization.
2. Wash with buffer solution.
3. Antigen retrieval with activation solution specialized for the equipment at 95 °C for 30 min.
4. Wash with buffer solution.
5. Delayed inhibitor.
6. Wash with buffer solution.
7. Primary antibody at 37 °C for 30 min.
8. Wash with buffer solution.
9. Streptavidin-biotin-peroxidase kit with 3,3′-diaminobenzidine at 37 °C for 8 min.
10. Wash with buffer solution.
11. Hematoxylin nuclear counterstaining at 37 °C for 8 min.
12. Wash with buffer solution.
13. Post-counterstain (lithium carbonate) at 37 °C for 4 min.
14. Wash with buffer solution.
15. Wash with water.
16. Dehydration with 100% alcohol and clearing with xylene.
17. Mount with Marinol.

57.2.2 Protocol for Immunocytochemistry Using Cytological Specimens

Immunocytochemical study is carried out using 95% alcohol-fixed smears from FNA. Alcohol fixation is used, followed by additional fixation with 20% phosphate-buffered formalin solution for 30 min and antigen retrieval with activation solution specialized for the equipment at 95 °C for 30 min. This procedure is followed by the steps from 2 to 17 in the protocol for tissue section immunohistochemistry. This protocol can also be used for staining decolorized Papanicolaou-stained smears and those from cell transfer technique [1–3].

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Table 57.1 Useful immunostaining markers for the diagnosis of thyroid diseases

Cellular structures/tumors/diseases	Positive markers	Negative markers
Normal follicular cells	Thyroglobulin (cytoplasm), thyroid transcription factor-1(nucleus), PAX8 (nucleus)	
Papillary carcinoma (PTC)	Cytokeratin 19 (cytoplasm), HBME1 (cell membrane)	
Cribriform morular variant PTC	β-Catenin (nucleus and cytoplasm), Estrogen Receptor (nucleus)	
Hyalinizing trabecular tumor	Ki-67(cell membrane)	Cytokeratin 19
Medullary (C cell) carcinoma	calcitonin (cytoplasm), carcino-embryonic antigen (cytoplasm), chromogranin A (cytoplasm), synaptophysin (cytoplasm)	Thyroglobulin
Poorly differentiated carcinoma	p53 (nuclei), Ki-67(nucleus), cytokeratins	
Anaplastic (undifferentiated) carcinoma	p53 (nuclei), Ki-67(nucleus), cytokeratins	Thyroglobulin, thyroid transcription factor-1
Intrathyroid thymic carcinoma	CD5 (cell membrane), p63 (nuclei), c-kit (cytoplasm)	Thyroglobulin, thyroid transcription factor-1
Metastatic renal cell carcinoma	CD10 (cell membrane)	Thyroglobulin, thyroid transcription factor-1
Malignant lymphoma	Lymphocyte markers (CD20 etc.)	Thyroglobulin, thyroid transcription factor-1, cytokeratins
Proliferation index (risk stratification)	Ki-67(nucleus)	

57.2.3 An Example of Fixation Effects on Immunohistochemical Results

As an example, MIB-1 (Ki-67) immunostaining of papillary thyroid carcinoma is presented to explain how the fixation causes difference in the results.

1. In the histopathological specimen of a papillary carcinoma, only few cells were positively stained (nuclear antigen), showing a MIB-1 labeling index of 1.3% (Fig. 57.1). However, in the alcohol-fixed cytological specimen from the same case, MIB-1 was negatively stained, showing a MIB-1 index of 0% (Fig. 57.2). When the alcohol fixation was followed by an additional step of formalin fixation (double fixation), MIB-1 was also negatively stained, showing a MIB-1 index of 0% (Fig. 57.3). On the other hand, when the specimen with alcohol fixation was followed by formalin fixation and antigen activation treatment, some tumor cells became positively stained with MIB-1 (Fig. 57.4), showing a MIB-1 index of 1.3%, which was identical to the histological specimen (Fig. 57.1). However, when the specimen with alcohol fixation alone went through the

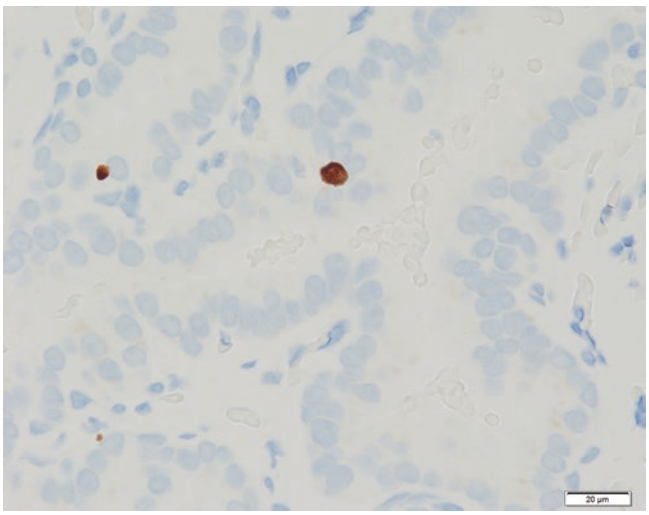

Fig. 57.1 MIB-1 staining of histological specimen with formalin fixation and antigen activation treatment (×400)

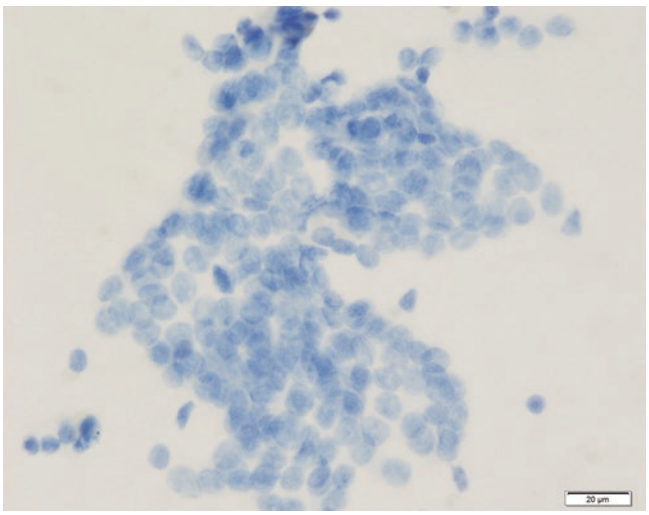

Fig. 57.2 MIB-1 staining of cytological specimen with alcohol fixation (×400)

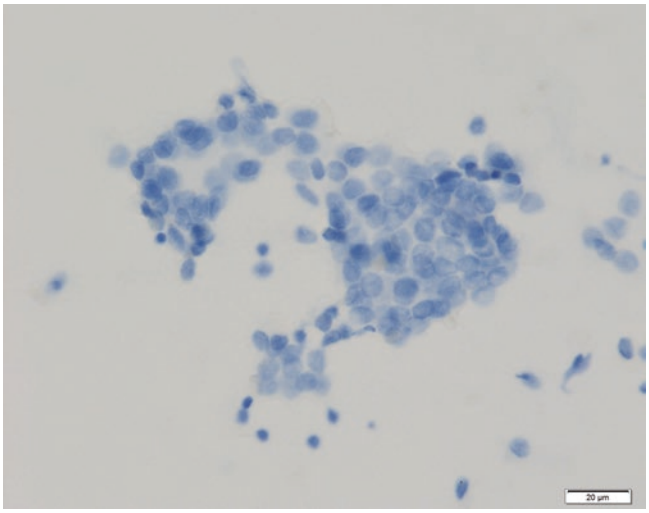

Fig. 57.3 MIB-1 staining of cytological specimen with alcohol and formalin double fixation (×400)

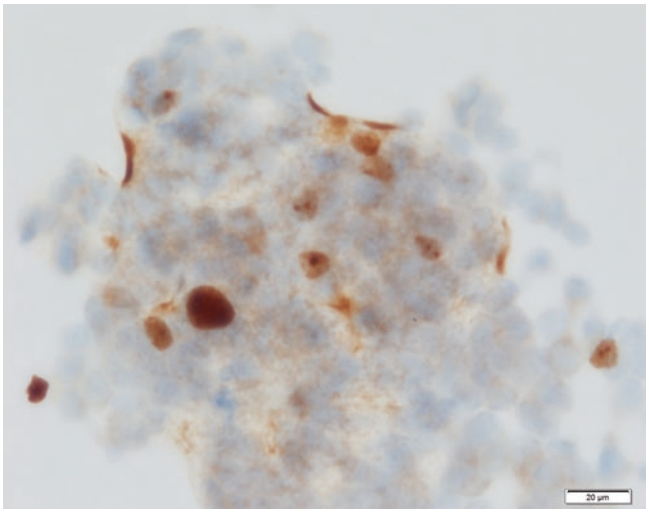

Fig. 57.5 MIB-1 staining of cytological specimen with alcohol fixation and antigen activation treatment. Note poor morphological details and higher non-specific staining (×400)

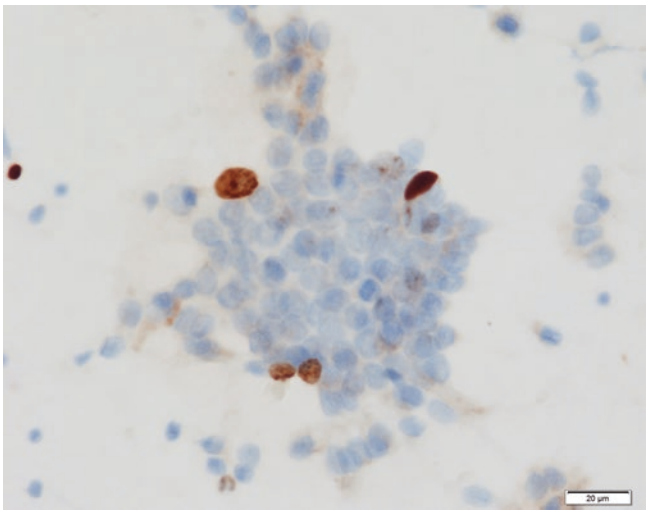

Fig. 57.4 MIB-1 staining of cytological specimen with alcohol and formalin double fixation as well as antigen activation treatment (×400)

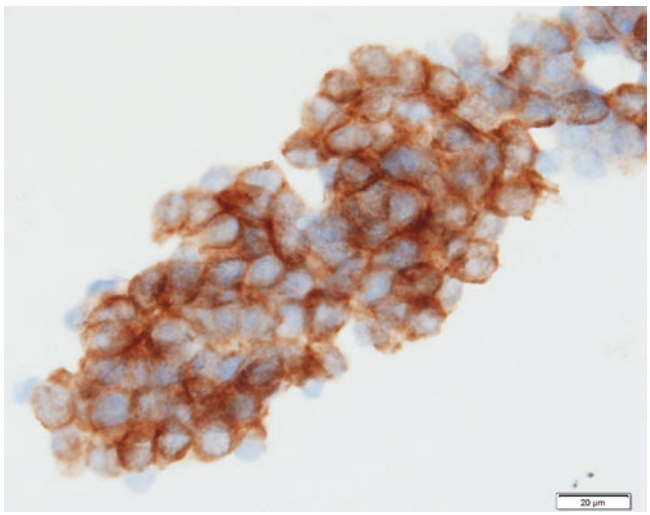

Fig. 57.6 CK19 staining of cytological specimen with alcohol and formalin double fixation as well as antigen activation treatment (×400)

activation treatment, MIB-1 was positively stained, but at the same time, the cellular details were demolished (Fig. 57.5). For other antigens of immunostaining, such as cell membrane and cytoplasm antigens, formalin fixation and antigen activation treatment often produce more favorable results. An example stained with CK19 is shown in Fig. 57.6.

57.3 Conclusion

For the purpose to obtain reliable and optimal results in immunostaining with cytological specimens, it is essential to formulate the standard staining conditions and to implement a regular protocol. Please refer to more immunohistochemical illustrations in other chapters listed in Table 57.2.

Table 57.2 Useful immunohistochemical markers in diagnosis of thyroid tumors and their cellular localizations

Markers	Chapters and illustration numbers	Antigen localizations
AE1/AE3 (pan-cytokeratin)	Chap. 37 (Fig. 37.11), Chap. 50 (Figs. 50.2 and 50.4), Chap. 64 (Fig. 64.17)	Cytoplasm
BRAF V600E	Chap. 64 (Fig. 64.15)	Cytoplasm
β-Catenin	Chap. 28 (Figs. 28.13 and 28.17)	Nucleus and cytoplasm
Calcitonin	Chap. 1 (Fig. 1.21), Chap. 39 (Fig. 39.2a), Chap. 40 (Figs. 40.7 and 40.10)	Cytoplasm
Carcinoembryonic antigen (CEA)	Chap. 40 (Fig. 40.11)	Cell membrane and cytoplasm
CD5	Chap. 41 (Figs. 41.5 and 41.9)	Cell membrane
CD10	Chap. 51 (Figs. 51.5 and 51.9)	Cell membrane
CD23	Chap. 37 (Fig. 37.10)	Cell membrane
CD30	Chap. 38 (Fig. 38.2)	Cell membrane
CD68	Chap. 33 (Fig. 33.3g)	Cytoplasm
Chromogranin A	Chap. 42 (Fig. 42.18)	Cytoplasm
Collagen type IV	Chap. 35 (Fig. 35.8)	Basement membrane (extracellular matrix)
Cytokeratin 19	Chap. 30 (Fig. 30.6), Chap. 31 (Fig. 31.10), Chap. 47 (Fig. 47.6f), Chap. 57 (Fig. 57.6), Chap. 69 (Case 5 Fig. 69.5a)	Cytoplasm
E-cadherin	Chap. 30 (Fig. 30.6)	Cell membrane
Estrogen receptor	Chap. 28 (Figs. 28.15 and 28.18)	Nucleus
GATA-3	Chap. 42 (Fig. 42.21)	Nucleus
HBME1	Chap. 31 (Fig. 31.9), Chap. 69 (Case 5 Figs. 69.2b and 69.5c)	Cell membrane
Ki-67 (MIB-1)	Chap. 34 (Fig. 34.10 and 34.13), Chap. 30 (Fig. 30.6), Chap. 40 (Fig. 40.12), Chap. 50 (Fig. 50.2), Chap. 57 (Figs. 57.1 and 57.4)	Cell membrane in hyalinizing trabecular tumors but in nucleus of others
PAX8	Chap. 42 (Fig. 42.20), Chap. 50 (Fig. 50.1)	Nucleus
Parathyroid hormone	Chap. 42 (Fig. 42.17)	Cytoplasm
PAX8	Chap. 42 (Fig. 42.20)	Nucleus
p53	Chap. 30 (Fig. 30.6), Chap. 48 (Fig. 48.11)	nucleus
p63	Chap. 50 (Figs. 50.2 and 50.3)	Nucleus
Thyroglobulin	Chap. 33 (Fig. 33.3f)	Cytoplasm
Thyroid transcription factor-1 (TTF1)	Chap. 42 (Fig. 42.19)	Nucleus

References

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