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Technical Note
PAXgene® Tissue System

Effect of epitope retrieval conditions on immunohistochemical staining of PFPE tonsil tissue with anti-human Ki-67 antigen (clone MIB-1)

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Introduction

Immunohistochemical staining is a commonly used technology for localization of antigens in histological sections. Most antibodies and related protocols were developed for sections of formalin-fixed, paraffin-embedded tissue (FFPE). Fixation with formalin often leads to chemical modification and crosslinking of proteins, leading to reduced immunoreactivity due to masking of epitopes. Lost immunoreactivity can be recovered by heating (98°C) fixed tissue in an aqueous retrieval solution. The most commonly used retrieval solutions are 10 mM citrate buffer, pH 6.0, and 10 mM Tris·Cl, 1 mM EDTA, pH 9.0.

In contrast to formalin fixation, the PAXgene Tissue System uses an alcohol-based 2-reagent system that preserves morphology and biomolecules without destructive crosslinking and degradation.

Ki-67 is a cell proliferation marker expressed during all phases of the cell cycle, but absent in resting cells. Cells stained with monoclonal mouse anti-human Ki-67 antigen display a nuclear staining pattern except in mitotic cells, where the chromosomes and the cytoplasm are labeled.

Study Design

A human palatine tonsil was divided into 2 parts, each with dimensions of approximately 4 x 10 x 10 mm. One part was fixed for 24 hours in neutral buffered formalin (NBF) and the other part was fixed in PAXgene Tissue FIX for 3 hours and stabilized in PAXgene Tissue STABILIZER for 24 hours before processing and embedding in paraffin.

Sections with a thickness of 4 µm were prepared from PAXgene Tissue-fixed, paraffin-embedded (PFPE) samples and from FFPE samples. Immunohistochemical staining was performed with monoclonal mouse anti-human Ki-67 antigen, clone MIB-1, from DakoCytomation (code no. M7240, 1:75 dilution in DakoCytomation® antibody diluent) in a labeled streptavidin-biotin (LSAB) assay, visualized with DAB+ (diaminobenzidine) and counterstained with hematoxylin. Heat-induced epitope retrieval was performed in a staining dish filled with target retrieval solution (citrate buffer, pH 6, DakoCytomation, code no. S1700; or Tris/EDTA buffer, pH 9, DakoCytomation, code no. S2368) at 70°C or 98°C for 10 or 20 minutes.

Results

According to the antibody supplier (DakoCytomation), optimal results for Ki-67 with FFPE sections of tonsil tissue are obtained using a 20-minute, heat-induced epitope retrieval step in citrate buffer, pH 6. Using the same conditions for PFPE sections, little or no staining was observed (Figure 1A). Since fixation with the PAXgene Tissue System does not lead to chemical modification of proteins, many antibodies work without heat-induced epitope retrieval in PFPE tissues (see the PreAnalytiX® Tissue Atlas for examples; www.preanalytix.com/knowledge/tissue/tissue-atlas). For Ki-67, however, there was no staining of PFPE tissue without epitope retrieval (Figure 1B). In contrast, intense nuclear staining was observed with epitope retrieval solution containing Tris/EDTA buffer, pH 9 (Figure 1C), even when less harsh epitope retrieval conditions were used (Figure 1D). In addition, with 10 minutes heating at 70°C a more intensive counterstaining was observed compared with 20 minutes at 98°C.

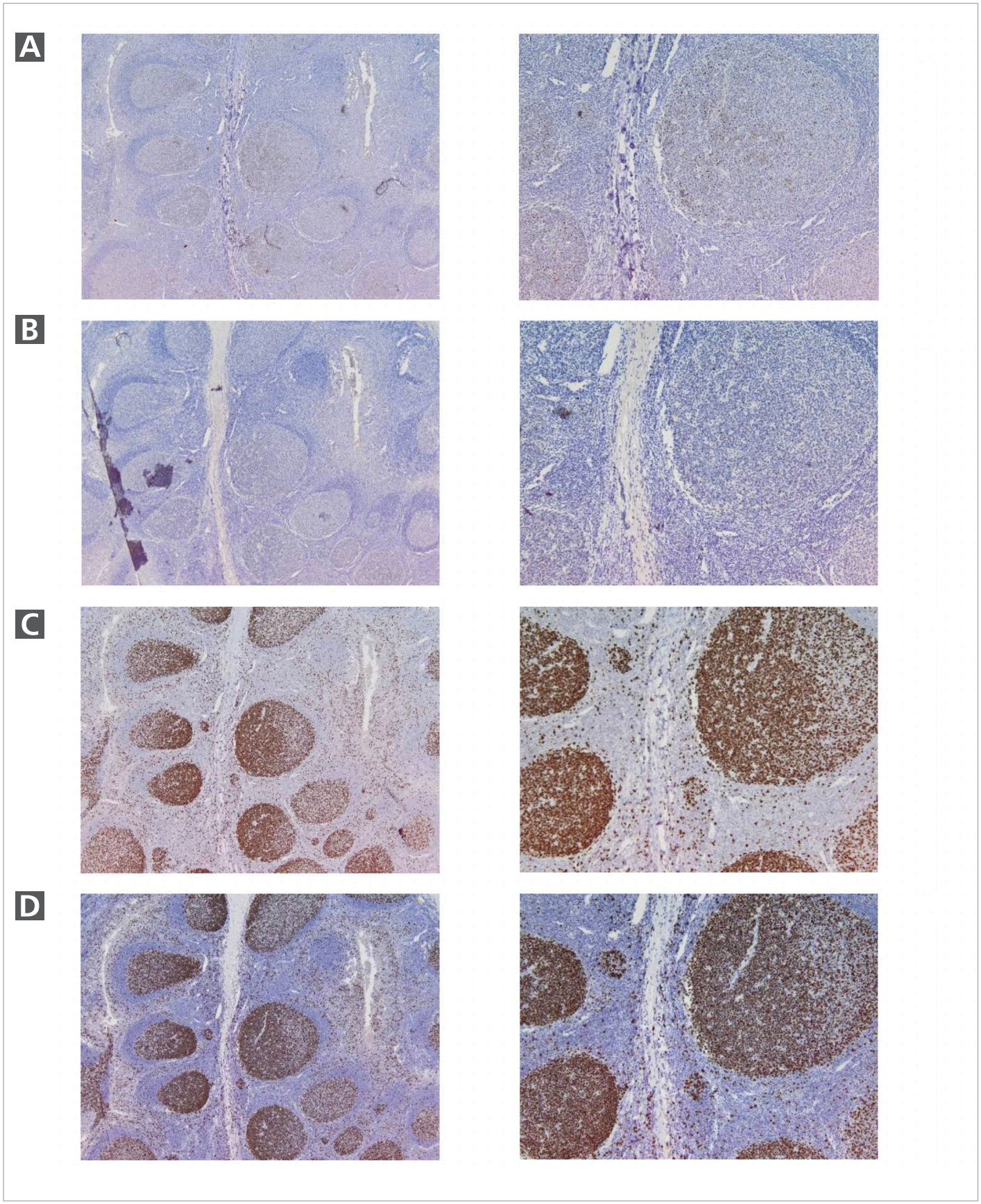

Figure 1. Optimization of epitope retrieval steps for immunohistochemical staining of Ki-67 in PFPE human tonsil tissue. 4 µm sections were stained with anti-human Ki-67 antigen, clone MIB-1; counterstaining for 1 minute with hematoxylin. Tissues are shown at 40x (left panel) and 100x (right panel) magnification. Epitope retrieval was performed for **A** 20 minutes at 98°C in citrate buffer, pH 6, **B** no epitope retrieval, **C** 20 minutes at 98°C in Tris/EDTA buffer, pH 9, or **D** 10 minutes at 70°C in Tris/EDTA buffer, pH 9.

A side-by-side comparison of PFPE and FFPE mirrored human tonsil tissue stained with identical reagents and staining conditions, but using the different heat-induced antigen retrieval steps, demonstrated equivalence for this specific antibody with regard to antigen localization, staining intensities and number of cells stained (**Figure 2**). Heating PFPE tissue for 10 minutes at 70°C in Tris/EDTA buffer produced staining intensities equivalent to those of FFPE tissue treated for 20 minutes at 98°C in citrate buffer.

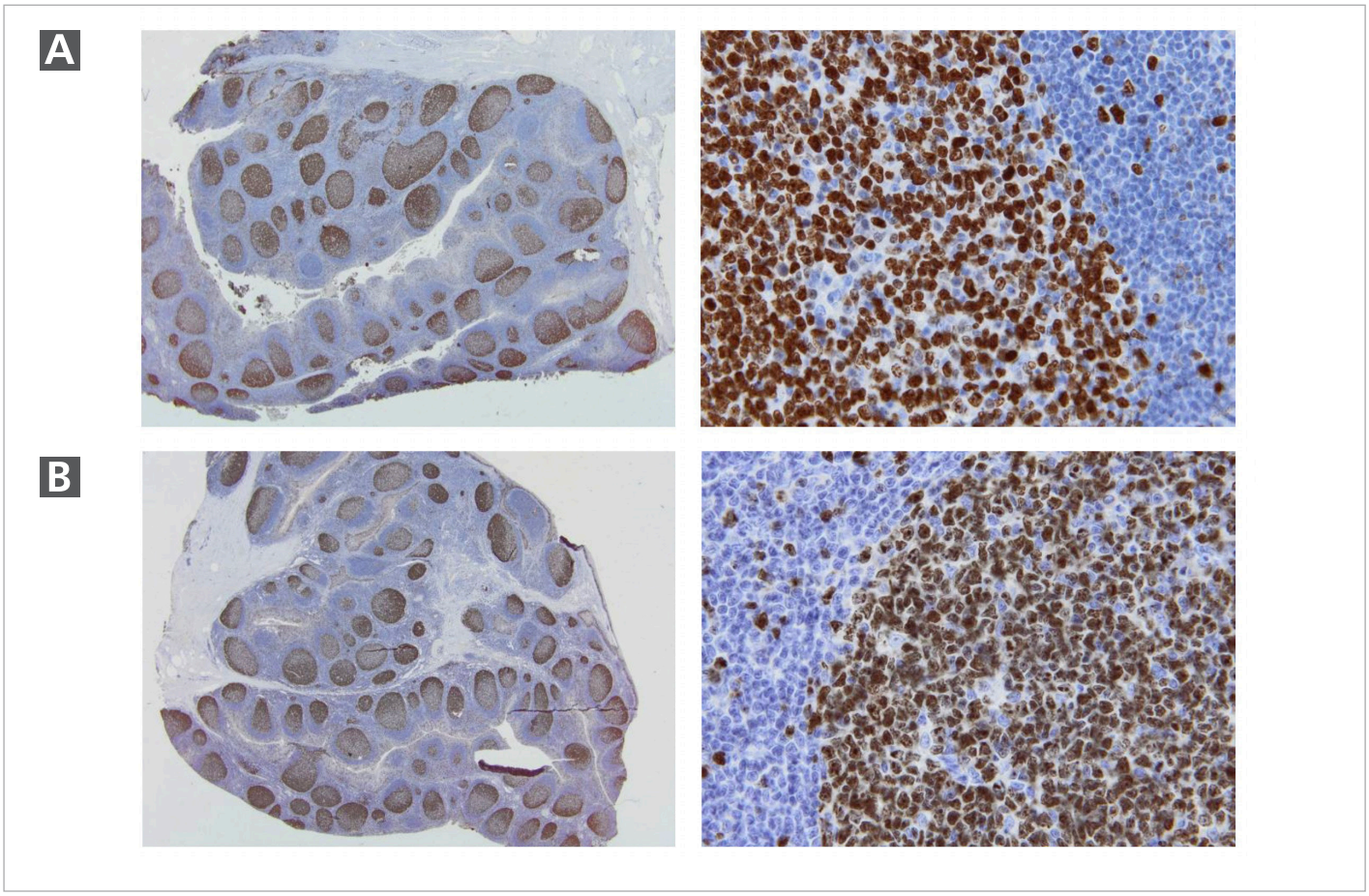

Figure 2. Immunohistochemical staining of mirrored samples from human FFPE and PFPE tonsil tissue. Sections (4 µm) were stained with anti-human Ki-67 antigen, clone MIB-1. Epitope retrieval was performed with PFPE tissues for 10 minutes at 70°C in Tris/EDTA buffer, pH 9, or with FFPE tissues for 20 minutes at 98°C in citrate buffer, pH 6. Tissues were counterstained with hematoxylin. Mirrored samples are shown at 12.5x (left panel) and 400x magnification for **A** FFPE tissues and **B** PFPE tissues.

Conclusions

Commercially available antibodies for immunohistochemistry applications sometimes require epitope retrieval for optimal staining of sections of PAXgene Tissue-fixed, paraffin-embedded (PFPE) tissue. This means that optimization of epitope retrieval should include variation of retrieval solution, incubation time and incubation temperature. After optimization sections of PFPE tissue can then be used for immunohistochemical staining.

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