PROPATH
THE FOCUS
Immunohistochemistry

Tissue Protection Immunohistochemistry

March 2002

by Rodney T. Miller, M.D., Director of Immunohistochemistry

Performing immunostains on small biopsies can be a challenging task, particularly when the lesion of interest is very tiny, a common occurrence in prostate biopsies. Since many laboratories routinely level paraffin blocks of needle biopsies, it is not uncommon for a small lesion to be present on the original H&E slides, but not present on deeper sections taken from the block for immunostaining. Nearly all pathologists have experienced this frustrating problem, which can be even more frustrating for the patient, who may need to endure the discomfort and expense of a repeat biopsy before a definitive diagnosis can be rendered.

There are several approaches that may be used to overcome this problem. Some laboratories routinely save intervening unstained paraffin sections between the various H&E levels, so that if immunostains are required, there will be satisfactory material available. Alternatively, immunostains can be performed on top of the H&E section, although if the H&E slides are mounted on regular (non-adhesive) slides, immunostains will usually fail secondary to unsatisfactory tissue adhesion to the slide.

Although we routinely use tissue transfer techniques to perform immunostains on previously-stained slides, this technique requires that the original material be on non-adhesive slides. Some months ago, Patty Kubier (our Immunohistochemistry Lab Supervisor), was confronted with a request for a high molecular weight cytokeratin immunostain on a prostate needle biopsy slide that had been previously placed on an adhesive slide, where a minute lesion was present only on the first H&E level. She devised a new technique which we termed "tissue protection immunohistochemistry", and published the method in the February 2002 edition of the *American Journal of Clinical Pathology*.

In order for tissue protection to be effective, it is critical that the original H&E slide be placed on an adhesive slide. This is becoming routine in many laboratories for

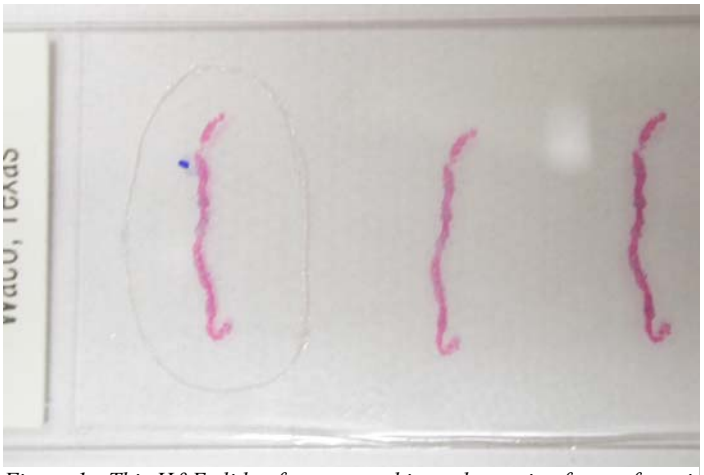


Figure 1: This H&E slide of a prostate biopsy has a tiny focus of atypical glands at the blue dot. This piece has been circled with a diamond-tipped pencil (on the back surface of the slide), and will subsequently be stained with high molecular weight cytokeratin.

Figure 2: The coverslip has been removed, and liquid coverglass media has been applied to the two H&E levels on the right. These two levels will be "protected" during the subsequent immunostaining procedure.

small needle biopsy specimens, where it is important to avoid any tissue loss. The first step in the procedure is to mark the section of interest to be stained (a diamond-tipped pencil works well), and remove the coverslip

from the previously-stained H&E slide (Fig. 1). After dipping in xylene, liquid coverglass medium is added to cover the sections that are NOT to be immunostained (thereby "protecting" those H&E sections, Fig. 2). At this point, the slide is subjected to pressure cooker antigen retrieval, which effectively destains the H&E. After removal from the antigen retrieval solution, the liquid coverglass medium has a somewhat cloudy appearance, but the liquid coverglass medium protects the other H&E levels from being destained during the antigen retrieval step (Fig 3). Routine immunostaining procedures follow, including a hematoxylin counterstain. During the final steps of dehydrating the slide in graded alcohols and xylene in preparation for coverslipping, the liquid coverglass medium dissolves in the xylene. At the end of the procedure, the slide has one immunostained section and adjacent H&E-stained sections (Fig 4). This makes correlating the appearance of the immunostain with the H&E very convenient.

If one chooses to routinely use adhesive slides for prostate needle biopsies, this technique can obviate the need for collecting and storing intervening unstained paraffin sections, and it has the advantage of not requiring a separate H&E destaining step. We have used this technique many times at ProPath with great success. The

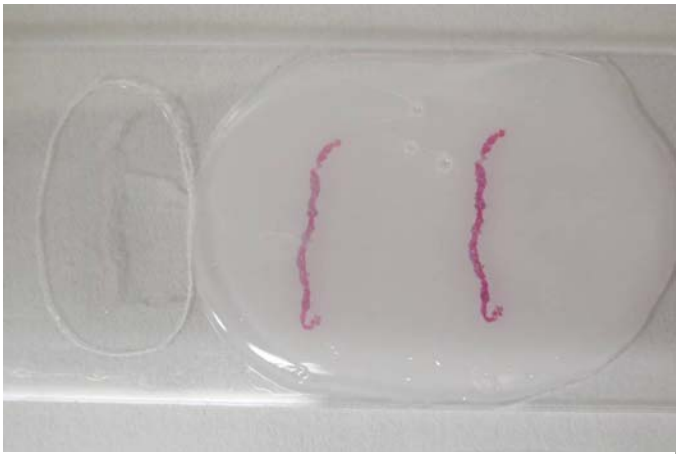
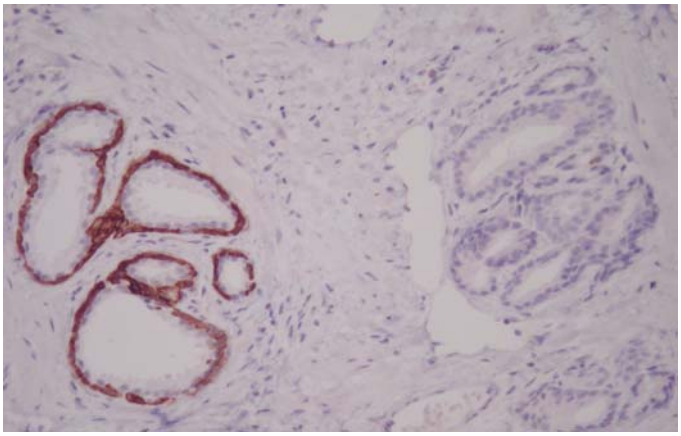


Figure 3: The pressure cooker antigen retrieval step has de-stained the left-most section, but the 2 H&E levels on the right have been "protected" from de-staining.

beauty of this technique is that it can be performed in any laboratory, can contribute to cost savings, and of greatest importance, it can decrease the number of repeat biopsies that patients may require to reach a definitive diagnosis.

This technique is not limited to prostate biopsies, and we have used it effectively to immunostain minute lesions from other organs as well, including a tiny focus of metastatic carcinoma in an endomyocardial biopsy, as well as, minute lesions in breast needle biopsies that disappeared in the deepest H&E levels. Hats off to Patty Kubier for coming up with this great idea!

Figure 4: Completed TPI immunostain, with the left-most section having a high molecular weight cytokeratin immunostain, and the right two sections stained with the original H&E. Below is the photomicrograph, showing staining of basal cells on the left, but absence of basal cells in the atypical focus (right).



REFERENCE:

Kubier P, Miller, RT: Tissue Protection Immunohistochemistry (TPI): A Useful Adjunct in the Interpretation of Prostate Biopsies and Other Selected Cases Where Immunostains are Needed on Minute Lesions. *American Journal of Clinical Pathology 117:194-198, 2002.*

Rodney T. Miller, M.D.
Director of Immunohistochemistry
(214) 237-1631
Fax: (214) 237-1770
rmiller@propathlab.com

Prior editions are available on our website.

PROPATH

8267 Elmbrook Drive, Suite 100 • Dallas, Texas 75247-4009
(214) 638-2000 • Fax: (214) 905-3457
www.propathlab.com