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 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.

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.
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 anti-
gen retrieval, which effectively destains the H&E. Af-
ter removal from the antigen retrieval solution, the liq-
uid coverglass medium has a somewhat cloudy appear-
ance, but the liquid coverglass medium protects the
other H&E levels from being destained during the anti-
gen retrieval step (Fig 3). Routine immunostaining
procedures follow, including a hematoxylin counter-
stain. During the final steps of dehydrating the slide in
graded alcohols and xylene in preparation for cover-
slipping, 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 sec-
tions (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 pros-
tate 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 tech-
nique many times at ProPath with great success. The

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 re-
peat biopsies that patients may require to reach a de-
finite diagnosis.

This technique is not limited to prostate biopsies, and
we have used it effectively to immunostain minute le-
sions 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 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.

REFERENCE:

Kubier P, Miller, RT: Tissue Protection Immunohisto-
chemistry (TPI): A Useful Adjunct in the Interpretation
of Prostate Biopsies and Other Selected Cases Where
Immunostains are Needed on Minute Lesions. Ameri-

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

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