M&M 2000 Expert's Session on Facility Management

Continued from preceding page

the staff to do a lot of these things. I am a one person EM facility for a medical college and half my time is spend teaching people how to use the confocal. We access the confocal facility via a combination lock and that works very well after training, if I am comfortable with the user. I am fortunate with the EM lab in that my Dean of Research is a very reasonable person and did not object when I declared myself empress of my facility and put up a note that said, "if you can't work here neatly you can't work here." I had occasion recently to call a full professor who held an endowed position. He had worked in the lab for three days and left it looking like a tornado had gone through it. I said, "This is not the way you found the lab when you came here on Tuesday. Please come down and restore it to its former condition." What is needed, as someone already said, is a good relationship with the upper management in order to do that. But having the lab clean when people walk in is important. I have people say "Wow, this place is really clean and neat!" and I say "Yes, and we try to keep it that way because otherwise it affects the quality of your samples." To get back to the issue of having your users understand not just the theory but the practicality of what they are doing, hopefully it clicks in that something seemingly as trivial as cleanliness and neatness affects the quality of their research and their results. ■

LOSS OF ANTIGENICITY ON STORED PARAFFIN SECTIONS

M A. Hayat, Kean University, Union, NJ
mhayat@turbo. Kean.edu

Sections of formalin-fixed and paraffin-embedded tissues are commonly used to detect antigens of diagnostic, therapeutic, or prognostic importance in patients with many types of cancers. Sections (~ 5 μm thick) cut from paraffin-embedded tissues are mounted on glass slides usually one or two days before immunostaining. In some cases, paraffin blocks are no longer available, and so immunohistochemistry must be performed on unstained slides that have been prepared sometime ago. It is not uncommon in both clinical and research laboratories to store unstained paraffin-embedded sections on glass slides at room temperature. Also, research laboratories that serve as reference centers for multi-institutional studies store slides for future use in order to rapidly return the tissue blocks to the originating institution. Such institutions want tissue blocks returned to them as soon as possible, as this may have medicolegal implications.

Storage of sections also becomes necessary when one marker study is proceeding and future marker studies need to be followed. Moreover, in such studies comparison of similar histologic feature among neighboring sections requires the availability of a large number of slides prepared from the same tissue block. Very small tissue samples, such as premalignant lesions of oral leukoplakia and dysplasia, require cutting a number of sections at the same time, so that the tissue block is cut conservatively, with only initial trimming of the meager sample.

Storage of slides at room temperature tends to result in temporary or permanent loss of antigenicity or antigen alteration, leading to false-negative immunostaining of tumor markers. Immunostaining of most antigens located in the nucleus, in the cytoplasm, or on the cytoplasmic membranes could be impaired by storing the paraffin slides. Only a few weeks of storage may induce diminished antigenicity, although the shortest duration of storage that has an adverse effect on antigenicity of most types of antigens is not known. The effect of storage of slides on some antigens is listed later.

A number of factors influence the temporary or permanent loss of antigenicity on paraffin sections stored on glass slides. The loss of antigenicity is gradual and consistent in most tissues. The most important factor that affects the antigenicity is the type and duration of fixation. The longer the fixation, the greater the masking or loss of the antigenicity. The tissue should be well-fixed, but not over-fixed. Generally, formaldehyde is preferred over glutaraldehyde because the latter excessively masks the antigens (Hayat, 2000a,b).

Not only the type of fixative and duration of fixation but also the duration of storage and the temperature of storage of sections influence the immunoreactivity. Prolonged storage of sections at room temperature causes diminished immunoreactivity. Grabau *et al.* (1998) have studied the immunoreactivity of estrogen receptor, Ki-67, p53, RBI, bel-2, E-cadherin, EGFR, and C-erbB2 on paraffin sections of lung carcinoma and breast cancer; the sections were stored for 3 years at -80°C, 4°C, or 20°C. In this study the immunoreactivity of all antigens except C-erb B2 was reduced at increasing temperatures of storage. Storage of sections at 4°C resulted in some diminished immunoreactivity that could be restored for all antigens, except for EGFR, using microwave heating. Quantitation of immunohistochemistry also

indicates that MIB-1 labeling index on sections (stored for 10 weeks) of the bladder carcinoma biopsies was significantly lower at 20°C than at 4°C ($p<0.0001$) (Wester *et al.*, 2000). Other factors that play a role in the loss of immunoreactivity during section storage include the type of tissue and the antigen, and the affinity of the antibody for the antigen.

The best approach to preserve antigenicity is to store the tissue deep in the paraffin block instead of storing the paraffin sections. Also, the sections should be processed immediately after they are cut. If the sections must be stored, they should be stored at 40C. Several other suggestions to minimize or prevent antigenicity loss on stored paraffin sections have been made in the published literature. Significant increase of immunoreactivity of p53, factor VIII, estrogen receptor, and bcl-2 on stored paraffin sections was reported in the tissue fixed with 10% buffered formalin supplemented with 70% ethanol (Jacobs *et al.*, 1996). However, ethanol is a poor preservative of cell structure (Hayat, 2000a).

The following immunohistochemical studies demonstrate diminished immunostaining of a wide variety of antigens on paraffin sections stored for a range of durations.

Intensity of immunostaining of chromogranin, CD3, and estrogen receptor has been reported to be decreased on paraffin sections of breast cancer, lymphoma, and neuroendocrine tumors stored for 3-12 months at room temperature (Bertheau *et al.*, 1998). Prioleau and Schnitt (1995) compared the immunostaining of p53 (in mammary ductal carcinoma using two antibodies, PAb 1801 and D07) on sections stored for two months at room temperature with that on freshly-cut sections from the same paraffin block and stained simultaneously. As excepted, the immunoreactivity was weaker on the stored sections. Similarly, loss of immunohistochemical staining has been observed on prostatic needle biopsy sections stored for a maximum period of 4 years using antibodies against androgen receptor, Ki-67, CD-44, and p27 antigens (Vis *et al.*, 2000). Some loss of immunoreactivity of the Ki-67 antigen in the colon tissue occurred when the cut sections were not immunostained within approximately one week using MIB-1 antibody (Holt *et al.*, 1997). In this study maintenance of sections at 4°C and away from the light did not prevent such a loss of immunoreactivity.

A considerable decrease in p53 antigen staining intensity ($p= 0.039$) but little decrease in the percentage of positively (after antigen retrieval) on sections of the head and neck squamosa cell carcinoma and non-small-cell lung carcinoma have been reported using slides stored for as long as 48 months at room temperature; the sections were cut from 4 to 25 years old paraffin blocks (Shin *et al.*, 1997). However, according to this study, this problem can be circumvented by using an antigen retrieval method. These authors conclude that the use of stored sections for p53 immunostaining is acceptable, provided staining intensity is not the only study parameter. Nevertheless, how long the sections can be stored and still achieve optimal immunostaining of p53 is yet to be determined.

It has been reported that the archival brain tissue stored for decades maintains its immunoreactivity. The strong resistance of senile plaques and neurofibrillary tangles to digestion and solubilization allows the preservation of immunoreactivity, for example in the Alzheimer's disease tissue stored in formalin or paraffin for as

Continued on following page

Loss Of Antigenicity On Stored Paraffin Sections

Continued from preceding page

long as ten years, but the immunoreactivity is completelv lost after 30 years in formalin (Dwork *et al.,* 1998).

In this study the sections were pretreated with 88% formic acid for 5 mm before immunohistochemistry using antibodies Ab 39, 4G8, or anti-ubiquitin. No attempt was made to use heat treatment for antigen retrieval. Also, no information is available regarding the effect of section storage on the immunoreactivity in these archival tissues.

Intraobserver, interobserver, or interlaboratory immunohistochemical inconsistensies are, in part, due to different durations of section storage. Therefore, in order to increase the reliability and quality of immunohistochemical studies, antigen alterations on unstained, stored paraffin sections should be taken into account. ■

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