Letters to the Editor

CORRESPONDENCE RE: VARMA M, LINDEN MD, AMIN MB. EFFECT OF FORMALIN FIXATION AND EPITOPE RETRIEVAL TECHNIQUES ON ANTIBODY 34βE12 IMMUNOSTAINING OF PROSTATIC TISSUES. MOD PATHOL 1999;12:472–8.

To the Editor: We read with interest the study by Varma *et al.* (1) on the effects of fixation and tissue pretreatment (*i.e.*, use of heat-induced epitope retrieval [HIER] techniques) on the reliability of the use of antibody clone 34βE12, directed against high-molecular-weight cytokeratins (HMWCK), to identify the outer cell layer in prostate epithelium. However, the accompanying editorial by Ramnani and Bostwick (2) seems to represent an attempt to derive a conclusion neither suggested nor demonstrated by the cited study of Varma *et al.*

Ramnani and Bostwick concluded that laboratories should "exercise caution" in the application and interpretation of results using 34βE12 in distinguishing benign from malignant prostate glands. The study of Varma *et al.*, however, is purely a technical one and did not address the issue of sensitivity and specificity of the finding of loss of HMWCK-positive cells in prostate tissue. It is important, in our judgment, to separate the technical from clinical questions, although we disagree with Drs. Ramnani and Bostwick on both counts.

Ramnani and Bostwick noted in their editorial that "we and others have noted that this antibody is temperamental and prone to variability in staining," but Drs. Ramnani and Bostwick did not mention whether this "temperamental" performance incorporates the HIER pretreatment scheme suggested by Varma *et al.*; certainly the data presented by Varma *et al.* contradict this statement. We could list scores of clinically useful antibodies, the performance of which could be characterized as "temperamental and prone to variability in staining*" if inappropriate or inadequate HIER techniques are performed before their use.* Antibodies to chromogranin A, for example, without adequate HIER, can be an unreliable marker of neuroendocrine carcinomas, but with appropriate HIER, their sensitivity for these tumors is well in excess of 90%. Thus, antibody 34βE12 is no different from other antibodies in this regard, and Varma *et al.* have provided us with useful technical guidelines to optimize antibody use. Furthermore, Varma *et al.* offered reassuring evidence that although prolonged formalin fixation can result in a progressive decrease in the immunostaining intensity with antibody 34βE12, this becomes a significant problem only after formalin fixation of more than 1 week. As noted by Varma *et al.*, "Such a time frame would be unusual in the diagnostic setting of routine surgical pathology practice." Thus, from a technical standpoint, the evidence, which we can corroborate from our own unpublished studies, suggests that antibody 34βE12 can be a very reliable diagnostic reagent.

With respect to the clinical applications of this antibody, Ramnani and Bostwick noted that "despite these attempts to optimize anti-keratin 34βE12 immunoreactivity, the experience of the past 15 years has taught us that this immunohistochemical marker for prostate cancer has significant shortcomings and that a more reliable marker is needed." Whose experience are Drs. Ramnani and Bostwick summarizing? Certainly it has not been our experience, and even before the use of the HIER technique as recommended by Varma *et al.*, many published studies have come to the very opposite conclusion—that judicious use of antibody 34βE12 is a cost-effective and even "indispensable" tool in confirming and establishing the correct diagnosis in questionable foci seen in prostate biopsies in the everyday practice of surgical pathology (3–7).

Addressing the issue of specificity, the finding of rare HMWCK-positive cells in prostate cancer nests, as illustrated by Varma *et al.*, is well taken but does not pose a serious problem to clinical interpretation. As noted by Varma *et al.*, "HMWCK immunostaining in malignant glands differs quantitatively and qualitatively from non-neoplastic glands." The rare presence of aberrant expression of HMWCK in prostatic carcinoma was confirmed by Yang and Epstein (8), who also noted that despite this finding, "this marker remains a very useful adjunct in the diagnosis of prostate cancer." Addressing the issue of sensitivity, as previously reported in abstract form by Varma *et al.* (9), a lack of 34βE12 immunostaining has been found around benign prostate lesions in 5% of 301 cases. It is impossible to derive meaningful numbers regarding antibody sensitivity in the absence of optimal tissue pretreatment: clearly, Varma *et al.* have provided the technical data needed to maximize the sensitivity of antibody 34βE12 for detecting the presence of the outer cell layer in benign prostatic glands.

Drs. Ramnani and Bostwick have also provided a list of potential alternative "basal cell specific" proteins, but many of these can be discarded out of hand: to name just a few, markers such as Ki-67 and PCNA identify nuclear cell proliferation–related proteins that would not be able to distinguish between cell type. Although one cannot predict what markers are yet to be discovered, clearly it will be difficult to come up with a marker that more accurately and reliably distinguishes prostatic carcinoma from normal and hyperplastic prostate tissue than 34βE12.

Drs. Ramnani and Bostwick are uncomfortable with the fact that the use of antibody 34βE12 relies on "the absence of staining to confirm the diagnosis of prostate cancer, unlike most immunohistochemical stains." (Of course, this potential problem would apply to any new putative basal cell–specific markers suggested by Drs. Ramnani and Bostwick.) However, in the daily practice of diagnostic immunohistochemistry, other markers are routinely assessed by their absence as well as their loss (*e.g.*, the evaluation of estrogen and progesterone receptor expression in breast cancer). A particularly helpful feature in prostate biopsies, even most needle core biopsies, is the presence of adjacent normal tissue that can serve as a positive internal control.

Finally, the editorial also stated that "antigen retrieval methods unmask epitopes that are otherwise inaccessible to the antibody due to the formation of covalent crosslinks between the aldehyde groups of formaldehyde and amino groups of proteins." This is still an assumption that is far from proved. In fact, there are accumulating data that argue against this statement. Studies performed by Morgan and colleagues (10), which we have confirmed, have demonstrated that the "recovery" of immunostaining provided by HIER techniques can be abrogated merely by adding divalent cations to the buffer containing the tissue sections. In addition, HIER techniques have salutary effects with some antigens (*e.g.*, the Ki-67 antigen) on tissue fixed in alcohol-based fixatives (*e.g.*, methacarn), which do not result in the formation of crosslinks.

There is no doubt that in the world of applied immunohistochemistry, no single marker comes close to perfection. The critical issue that should have been addressed in Drs. Ramnani and Bostwick's editorial is the clinical implications of the optimized HIER pretreatment outlined by Varma *et al.* Unfortunately, the editorial did not speak to this. Contrary to what Drs. Ramnani and Bostwick wrote in their editorial, we believe that 34βE12 is a very reliable reagent that has compelling clinical utility in the analysis of prostate cancer.

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CORRESPONDENCE RE: KAWANO N, INAYAMA Y, NAGASHIMA Y, MIYAGI Y, UEMURA H, SAITOH K, *ET AL*. DESMOPLASTIC SMALL ROUND-CELL TUMOR OF THE PARATESTICULAR REGION: REPORT OF AN ADULT CASE WITH DEMONSTRATION OF EWS AND WT1 GENE FUSION USING PARAFFIN-EMBEDDED TISSUE. MOD PATHOL 1999;12:729–34.

To the Editor: I enjoyed reading the article by Kawano *et al.*, showing that a desmoplastic small round cell tumor of the paratesticular region shows similar phenotype and genotype to its abdominal counterpart. However, I must take issue with the legend to Figure 3B, which describes a "desmosome-like structure" seen by electron microscopy. It appears to me that the illustration actually shows a true desmosome, with these features as delineated by Ghadially (1): a widened intercellular gap filled by dense material, attachment plaques on the cytoplasmic faces, and tonofilaments converging on the plaques. Indeed, in the discussion of the same paper, the authors stated that "some tumor cells showed dense core granules, *desmosomes*, intermediate filaments, and microtubules" (emphasis added). It is important to distinguish between true desmosomes and desmosome-like structures, because the former are of diagnostic significance, whereas the latter are found in a wide variety of tumors other than lymphoma. It seems critical for an official journal of the United States and Canadian Academy of Pathology to maintain the strictest criteria in nomenclature, to avoid misleading pathologists-in-training and indeed even practicing pathologists.

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