

PROPATH

THE FOCUS

Immunohistochemistry

True Positive vs. False Positive Staining

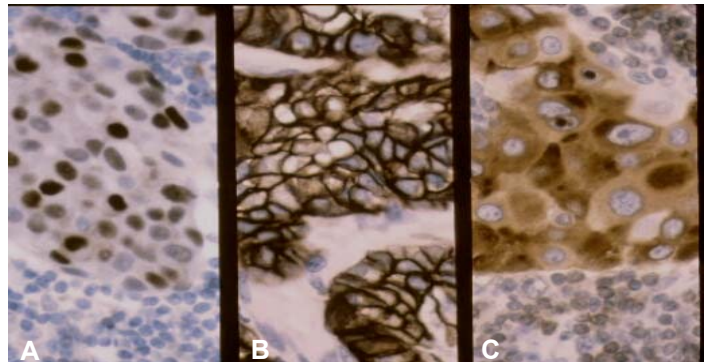
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by Rodney T. Miller, M.D., Director of Immunohistochemistry

Immunohistochemical stains are indispensable for problem solving in diagnostic pathology. In order to evaluate them appropriately, it is critical to be aware of the characteristics of a **true positive stain** and a **false positive stain**. A "true positive" stain shows chromogen deposition in cells or structures that truly contain the antigen of interest. In contrast, a "false positive" stain is one where the chromogen is localized to cells or structures that in reality lack the antigen of interest. When using diaminobenzidine (DAB) chromogen with peroxidase-based detection systems, this problem can be expressed simply as "all that is brown is not real". Stains in which the entire section (both tumor and intervening non-neoplastic stroma) is a dark "muddy" brown are easily recognized by nearly any observer as falsely positive. Fewer observers realize that false positive staining commonly occurs in a different situation: where the tumor cells are strongly stained but the background non-neoplastic stroma is "clean" and free of chromogen deposition.

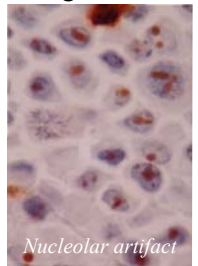
Before discussing this latter type of false positive staining (where the chromogen is paradoxically exquisitely localized to the cells of interest), it is useful to keep in mind the patterns of reactivity seen with true positive stains. One feature characteristic of true positive stains is **cell-to-cell heterogeneity**. This refers to the fact that the deposition of the reaction product often varies in intensity among cells, and also often within parts of a single cell. Another pattern of true positive immunoreactivity is that of **distinct crisp cell membrane reactivity**, which is only rarely seen in false positive staining artifacts. Immunoreactivity restricted to **cell nuclei** (e.g., estrogen receptor) is another type of true positive reactivity, but in a true positive nuclear stain one often can still appreciate some degree of cell-to-cell heterogeneity. A potential trap in interpretation of nuclear staining is spurious reactivity of "optically clear" nuclei in gestational endometrium (and also in some pulmonary blastomas), secondary to binding of endogenous biotin present in these nu-

clei. I have also seen artifactual false positive staining of cell nuclei in some cases (particularly with CD15) in areas adjacent to necrotic tissue, but in these instances the intensity of the label is uniform and often somewhat indistinct (i.e., it lacks cell-to-cell heterogeneity and may appear "muddy"). On occasion a false positive nuclear stain may also be seen when heavy-metal fixatives are



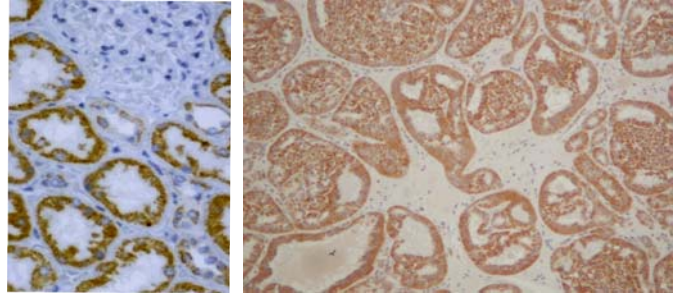
Patterns of true positive immunostaining: Nuclear reactivity for ER in a breast cancer (A); Crisp cell membrane reactivity for HER2 in a breast cancer (B); Cytoplasmic reactivity for GCDPF-15 in a metastatic breast carcinoma involving lung (C); Note cell-to-cell heterogeneity, typical of true positive immunostains.

used, such as B-5 or Zenker's, and I have also seen intensely positive false positive nuclear staining artifacts when employing strong acids as decalcifying agents or as epitope retrieval solutions. For this reason it is important to know the expected pattern of immunoreactivity (nuclear, cytoplasmic, or membrane) for the antigen of interest. False positive staining of nucleoli may be seen with CD20, CD7, and BCL-10, a finding of no diagnostic significance.



An important trap alluded to above is that false positive immunoreactivity may be very intense and can be precisely localized to the cells of interest (usually tumor

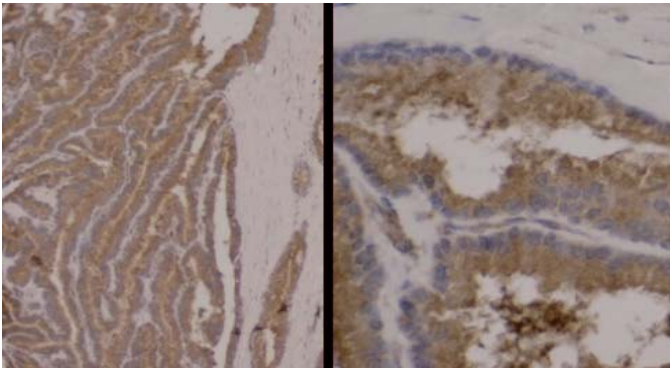
cells), with no staining of the background stroma observed. However, this type of reactivity can be readily recognized if the observer is aware that this type of false positive staining characteristically LACKS cell-to-cell heterogeneity. In these instances, the cells display a uniform cytoplasmic "blush" that appears "muddy" and indistinct, lacking the crisp heterogeneous quality of a true positive stain. This pattern of false positive staining is usually secondary to the use of an inappropriately concentrated titer of primary antibody, or to using a (so-called) "predilute ready-to-use" antibody at an inappropriate titer (i.e., used as supplied without testing further titers). **THERE IS NO SUCH THING AS A PREDILUTE READY-TO-USE ANTIBODY!**



Endogenous biotin artifact in proximal convoluted tubules of kidney (left) and renal oncocytoma (right). These are negative control specimens, stained using an avidin-biotin procedure, performed without blocking endogenous biotin. Despite the clean stroma and exquisite localization to the cells of interest, the lack of cell-to-cell heterogeneity is a clue that this staining is spurious in character.

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Classic pattern of false positive immunoreactivity. Even though the chromogen is exquisitely localized to tumor cells and background stroma is clean, the lack of cell-to-cell heterogeneity and the "muddy" appearance suggests false positive staining. This pattern is often due to an inappropriately concentrated primary antibody titer. This slide is an HMB-45 immunostain on a papillary thyroid carcinoma, done at the manufacturer-suggested titer, which obviously was too concentrated. This reactivity totally disappeared with an appropriate primary antibody titer.

Binding of avidin components to endogenous biotin present in tissues or cells may also cause false positive staining when avidin or streptavidin-based detection systems are used, particularly when effective antigen retrieval techniques are used. This is most frequently observed in liver and kidney (since these tissues have numerous mitochondria and therefore a high biotin content), where it appears as granular cytoplasmic chromogen deposition that is noticeable on the negative control specimen, as well as on the patient specimen **(provided that the negative control was subjected to the same epitope retrieval procedures)**. This problem can be economically and effectively dealt with by routinely employing steps to block endogenous biotin activity in the staining method, using dilute egg whites as a source of avidin and reconstituted 5% dried milk or 0.2% biotin in PBS as sources of biotin.

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