

Endogenous Biotin Artifact

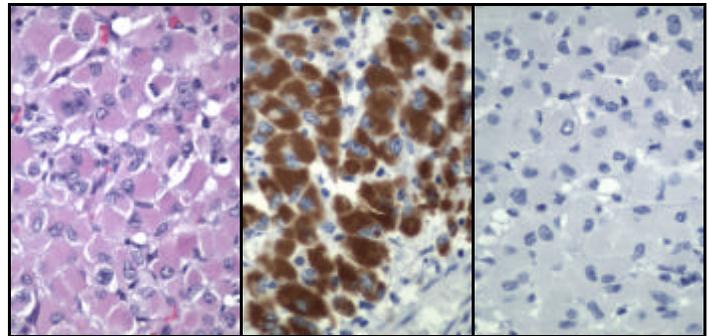
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In the past few months, several cases were referred to us for review in which endogenous biotin artifact led to difficulties in initial interpretation of the cases. As such, it is apparent that not all laboratories that employ avidin-biotin-based detection systems incorporate methods for blocking of endogenous biotin. In the hopes of increasing the recognition of this artifact and decreasing its occurrence, this month's Focus discusses and illustrates endogenous biotin artifact and offers approaches to abolish this problematic and potentially significant artifact.

Biotin is a vitamin that serves as a cofactor for enzymes involved in carboxylation reactions. In human tissues, most biotin is associated with the mitochondrial enzyme pyruvate carboxylase. Therefore, cells that contain numerous mitochondria have high amounts of endogenous biotin. As might be predicted, cells that contain numerous mitochondria (such as proximal convoluted tubules of the kidney, hepatocytes, and oncocytomas) have the greatest potential for showing this artifact. However, we have personally observed significant endogenous biotin artifact in many other types of cells, particularly carcinomas, from a wide variety of primary sites.

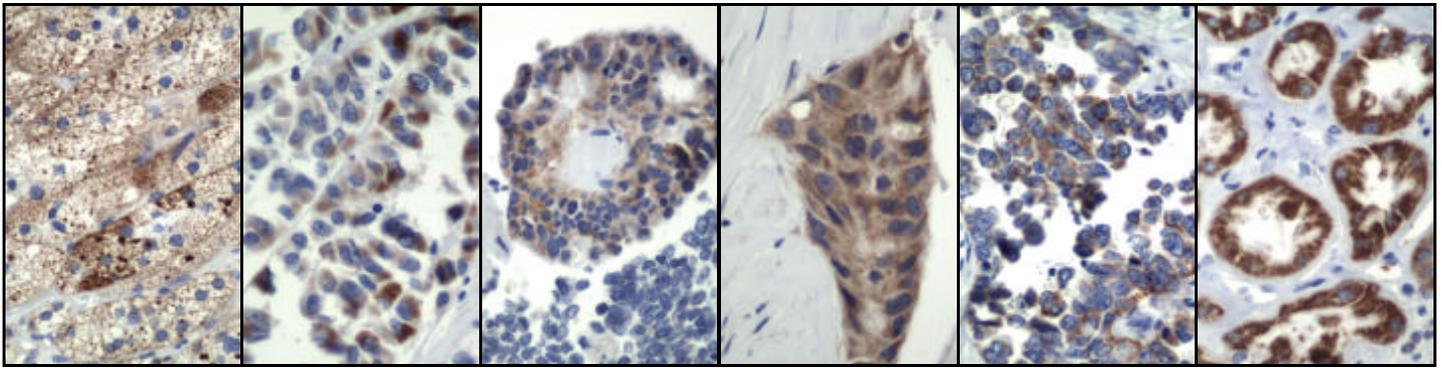
Many immunohistochemistry laboratories employ a detection system based on the strong binding of biotin to avidin (a protein found in egg whites) or streptavidin. One molecule of avidin can bind 4 biotin molecules, and this binding is very strong and for practical purposes irreversible. In these avidin-biotin-based methods, after application of the primary antibody, the secondary (or "link") antibody used is typically labeled with biotin. The subsequently employed detection reagent consists of a complex of avidin, biotin, and labeling enzyme (most commonly peroxidase) in which some of the biotin-binding sites present on the avidin are empty, ready to bind any biotin that they subsequently encounter. When this detection complex is placed on the tissue section (which contains the biotin-labeled secondary



H&E (left) from biopsy of adrenal tumor of unknown origin. A CK20 stain (center) was performed with an avidin-biotin detection system, without incorporating steps to block endogenous biotin. The CK20 stain performed using a polymer-based detection system (right) was completely negative. This nicely illustrates endogenous biotin artifact in an adrenal oncocytoma.

antibody), the avidin in the detection complex binds to the biotin present on the secondary antibody, accounting for its localization to the target antigen of interest. However, since the avidin present in the detection complex has no way of distinguishing biotin present on the secondary antibody from endogenous biotin present in cells, the detection complex will also bind any endogenous biotin that it encounters, ultimately resulting in labeling of cells that do not necessarily contain the target antigen of interest.

Heat-induced epitope retrieval (a.k.a. antigen retrieval) techniques employ heating of tissue sections in a variety of solutions before application of primary antibody. These methods have dramatically improved results of immunostains in formalin-fixed paraffin-embedded material, since they markedly improve the sensitivity of detection of a wide variety of antigens, effectively "unmasking" targets of interest. Unfortunately, these techniques also markedly increase the detectability of endogenous biotin, thereby serving to "unmask" endogenous biotin as well. The degree of unmasking of endogenous biotin by antigen retrieval varies depending on the method used, but it can result in extremely intense labeling of cells.



Multiple examples of endogenous biotin artifact. All photographs are prostatic acid phosphatase immunostains performed using antigen retrieval and an avidin-biotin-based detection method, with no steps taken to block endogenous biotin. Tissues showing endogenous biotin artifact resulting in spurious staining are as follows: (from left to right): adrenal adenoma, metastatic melanoma, metastatic colonic adenocarcinoma, metastatic lung carcinoma, serous carcinoma of ovary, and normal kidney cortex. When steps were taken to block endogenous biotin (or when a polymer-based detection system was employed), there was no staining.

Since endogenous biotin artifact represents labeling of non-target elements, it is readily apparent on negative control slides, provided that the negative control immunostain has been subjected to the same antigen retrieval techniques as the slides treated with primary antibody. If the negative control slide is not subjected to antigen retrieval (and the slides treated with primary antibody are), any endogenous biotin artifact may be apparent only in the slides treated with primary antibody. Obviously in this situation, there is great potential for misinterpretation of endogenous biotin artifact as "true" immunoreactivity. Therefore, **it is critical that negative control slides be subjected to the same antigen retrieval techniques as those slides that receive primary antibody.**

Fortunately, there are simple methods available to effectively abolish this artifact. Following performance of the antigen retrieval steps, tissue sections can be layered with a solution of avidin (which will bind to any endogenous biotin present in the tissue). After rinsing, a solution of biotin is then placed on this tissue sections, and this biotin will saturate any empty biotin-binding sites that are present on the avidin. Following another rinse, the primary antibody is added to the tissue sections, and the remainder of the immunostain procedure is performed as usual. Since avidin is expensive (over \$3500 per gram), employing commercial avidin-biotin blocking kits can add significant expense to the staining procedure. However, we have shown in our laboratory that a dilute solution of egg whites (or egg substitute) can be used as a source of avidin, and skim milk (or reconstituted powdered milk) can be used as a substitute for biotin, with results equivalent to those obtained with commercial avidin-biotin blocking kits. Alternatively, employing a polymer-based detection system effectively eliminates any problems with endogenous biotin, since polymer-based methods do not incorporate avidin and biotin in the staining method.

Because endogenous biotin artifact can result in intense staining exquisitely localized to tumor cells of interest (that happen to contain endogenous biotin), if not recognized, it can readily lead to misinterpretation of a tumor's immunophenotype, and could very easily result in a misdiagnosis. For this reason, **all diagnostic laboratories that employ avidin-biotin-based detection systems and heat-induced epitope retrieval should routinely include methods for blocking of endogenous biotin as part of their staining procedure, performed with every immunostain done for diagnostic purposes.**

References:

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