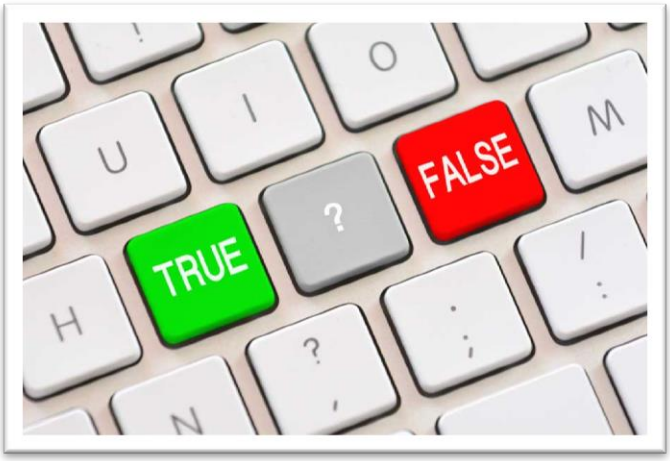
High-Quality IHC Reagents Reduce False Positives

Posted by Arun Kumar



Immunohistochemistry (IHC) is a widely used biological technique, which allows the researcher to analyze the anatomy of the tissue of interest and to visualize the distribution, localization, and intensity of expression of a specific antigen or cellular components in health and disease. It is an important tool for scientific research as evidenced by an exponential increase in publications in IHC applications over the last couple of decades. Many improvements in the field of IHC have greatly expanded the application of this technique. Pathologists can now make definitive diagnoses and treatment decisions based on cellular presence or absence of a particular antigen. Robust methods of generating antigen-specific antibodies and automation of the assay are but a few of the advances that have contributed to the successful application of IHC in differential diagnoses, which were not possible by conventional analysis with hematoxylin and eosin (H&E) staining.

Steps in IHC Detection

There are three major steps in IHC: (A) binding of the primary antibody to a specific antigen, (B) formation of an antibody-antigen complex following the addition of a secondary enzyme-conjugated detection antibody and finally, (C) presence of a chromogenic substrate, which in the presence of an enzyme leads to the generation of a colored deposit at the site of the antibody-antigen complex. When performed manually, IHC can be a lengthy process that is prone to errors. In order for a histopathology laboratory to deliver IHC results to clinicians in a consistent and timely manner, there is a need to process hundreds of samples efficiently. Cost-saving and reliable high-throughput staining capabilities are therefore essential for histopathologists and researchers alike. Automated platforms with slide staining features have been developed to execute time-consuming processes. They offer the advantages of programming reagent additions in a pre-determined manner as well as rinsing/washing in between periods of incubation. These developments have allowed the end user to optimize staining conditions in terms of primary antibody dilution, detection reagents, and chromogens for each individual marker with the principal goal of achieving maximum sensitivity and specificity, minimizing non-specific binding while reducing time to result and cost. There are

many commercially available kits and automated platform employed in diagnostic IHC facilities. The automated systems from Leica and Ventana are widely used. As they are "open systems," they offer the flexibility to use detection reagents and chromogens of choice.

Identification of False Positives

It is well documented that variables such as antibody concentration, incubation time of the primary antibody and detection kit can provide both false-negative and false-positive results. The most commonly used catalytic enzyme in diagnostic IHC is horseradish peroxidase (HRP), which has some disadvantages such as susceptibility to interfering factors, unstable sensitivity, and high discrepancy among laboratories. Endogenous peroxidase activity is found in many tissues and can be detected by reacting fixed tissue section with 3, 3'-diaminobenzidine (DAB) substrate. Consequently, false positives are noticed due to inappropriate activity of the peroxidase in cells that do not contain the antigen of interest.
Professor Nuovo from Ohio State University Comprehensive Cancer Center performed a side-by-side comparison of HRP conjugate from Leica, Ventana, and Enzo Life Sciences on a Leica BOND-MAX platform. The study focused on surgical biopsy samples where histologic diagnosis was confirmed by hematoxylin and eosin (H&E) staining. Ki-67 and p16 are two markers commonly used by clinicians to distinguish human papillomavirus (HPV)-positive cervical intraepithelial neoplasia (CIN) from HPV-negative mimics of CIN. As expected, all CIN1 lesions were positive for Ki-67 and p16 with all three HRP conjugates. False positive were obtained in 29% and 50% of CIN mimics using the Leica and Ventana HRP conjugates, respectively. Using Enzo's POLYVIEW® PLUS HRP reagents, staining specificity was dramatically improved as mimics of CIN were found to be negative for both Ki-67 and p16. Similar experiments were performed looking at the expression of HER2/neu in triple-negative breast cancers, Ki-67 in leiomyomas, and HMB45 in metastatic thyroid cancer and melanoma. The author demonstrated that false positives ranged from 13 to 36% when using Leica and Ventana detection reagents while there was no false positive signal with Enzo's detection reagents regardless of the target and the tissue origin.

Conclusion

The results from this study points to the background staining issues as a causative factor leading to false-positives in IHC diagnostic application. A precise explanation for the observed high background cannot be advanced given the proprietary composition of HRP conjugates. One possibility for the superior performance of Enzo HRP conjugate could be that the formulation of Enzo's reagents minimizes non-specific binding to cells that do not contain the epitope of interest. Immunohistochemistry using antibodies to beta amyloid, alpha synuclein,

ubiquitin, huntingtin, polyglutamine, and others has become a routine tool for sensitive detection and quantification of these abnormal proteins in both human tissues and in experimental animals that are used to model some of the features of these diseases. This study suggests that false positive results can be minimized by considering the source of HRP conjugate. The POLYVIEW® PLUS IHC detection reagents enable stronger staining with significantly lower background.

Enzo Life Sciences offers histopathologists a comprehensive portfolio for Immunohistochemistry including antibodies, tissue microarray, retrieval reagents, detection reagents and a wide variety of chromogens allowing multi-color immunohistochemistry staining.