Compatibility of POLYVIEW® IHC Detection Reagents and HIGHDEF® IHC Chromogens with Automated IHC Slide Stainers

POLYVIEW® IHC reagent (mouse-HRP) (ADI-950-112)
POLYVIEW® IHC reagent (rabbit-HRP) (ADI-950-115)
HIGHDEF® IHC chromogen substrate (DAB, stable) (ADI-950-212)

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ABSTRACT

Immunohistochemistry is an important diagnostic, prognostic and research tool to analyze tissues of different origins and different species. However, IHC reagents are not always easily adapted to high-throughput staining methods. This application note demonstrated the use of Enzo Life Sciences' POLYVIEW® IHC detection reagents and HIGHDEF® IHC chromogen with Dako Autostainer Link 48 and Biocare Medical intelliPATH™ FLX as platforms to stain key biomarkers used in both fundamental and clinical cancer research.

INTRODUCTION

Immunohistochemistry (IHC) is a widely used biological technique allowing the end user not only to analyze the anatomy of the tissue of interest but also to visualize the distribution, the localization and the intensity of the expression of a specific antigen or cellular components in tissue sections. It can be summarized in three major steps: (A) the binding of the primary antibody to a specific antigen, (B) the formation of an antibody-antigen complex following the addition of a secondary enzyme-conjugated detection antibody and finally, (C) the presence of a chromogenic substrate, which in contact of the enzyme will lead to the generation of a colored deposit at the site of the antibody-antigen complex. When done on a bench, it can be a lengthy and not always identically reproducible process. For a histopathology laboratory to deliver IHC results to the clinicians in a consistent and timely manner, it needs to be able to process hundreds of samples in a very short period of time. Researchers face, also a prerequisite to study dozens of specimen materials in order to get true statistical significance when establishing new biomarkers for a disease or a specific disease state. Cost-saving and reliable high-throughput staining capabilities are, therefore, of essence for histopathologists and researchers alike. Automated slide stainers have been specifically developed for such a complex and time-consuming process. They provide an experimental environment for reagents to react with the sample during timed periods of incubation, and are capable of applying unique reagents in a predetermined manner as well as rinsing/washing buffers in between periods of incubation. Systems, such as the Autostainer Link 48 from Dako or the intelliPATH™ from Biocare Medical, possess opened operating modes providing them with a flexibility in protocol programming and stain sets. They allow 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 being to reach maximum sensitivity and specificity, minimize non-specific binding whilst reducing time to results and costs (1).

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Nowadays, the efficient analysis of a tumor sample relies heavily on the expression analysis of tumor biomarkers by immunohistochemistry. Some of these biomarkers are especially relevant not only in diagnosis, but also in prognosis with different cancers featured with different panels of biomarkers. Several have now been validated for diagnostic and/or research use such as Bromodeoxyuridine (BrdU), cytokeratins, Ki67 or p53. Bromodeoxyuridine is a synthetic nucleoside and an analog of thymidine. Because of its non-radioactivity and non-myelotoxicity, it is commonly used by researchers conducting in vivo studies of cancer cell proliferation after infusion of the chosen animal model. Antibodies specific for BrdU can then be employed to detect the incorporated chemical by immunohistochemistry to indicate cells capable of rapidly and actively replicating (2). Cytokeratin proteins are integral components of keratin-containing intermediate filaments, which are localized in the intra-cytoplasmic cytoskeleton of epithelial tissue. They are involved in the organization of the cytoplasm, the support of the nucleus, and cellular communication mechanisms. The subsets of cytokeratin proteins expressed in an epithelial cell depends on the type of epithelium, the moment in the course of terminal differentiation and the stage of development. A classification can therefore be achieved by allocating a definite cytokeratin profile to specific epithelial cells and their malignant counterparts, carcinomas (3, 4). Ki67 is a nuclear protein and a key cellular marker for proliferation. It can be found in the nucleus through the different phases of the cell cycles going from the cell nucleus during G1, S and G2 to the surface of the chromosome during mitosis. It is used by histopathologists to determine the percentage of Ki67-positive cancer cells in a given sample, which often correlate with the prognosis, notably in cases of brain, breast and prostate carcinomas (5, 6). Finally, because of its function as a tumor suppressor gene, the transcription factor p53 has been described as “the guardian of the genome”. It has many anti-cancer mechanisms including the ability to arrest the cell cycle and initiate DNA repair in response to stress (e.g. DNA damage or hypoxia). It is also involved in apoptosis and inhibition of angiogenesis. Mutations in p53 lead to a conformational change in protein structure leading to an increased stability. P53 is, therefore, found in higher levels in malignant cells than in normal cells and very often studied as an antigen of interest within a panel of other biomarkers (7, 8).

The Beatson Institute for Cancer Research is one of Cancer Research UK’s core-funded institutes and as such, conducts a program of world-class sciences focused on the decryption of critical features of cancer cell behavior and the translation of these discoveries into new therapies and diagnostic/prognostic tools to help cancer patients. The institute delivers a range of histology and immunohistochemistry services for the research groups and large-scale IHC investigations can be undertaken thanks to top-of-the-range instruments including automated slide stainers. The main objective of this study was to look at the suitability of POLYVIEW® IHC detection reagents and HIGHDEF® IHC chromogens for these autostainer platforms and their potential in terms of sensitivity and quality of staining. Data obtained with Dako Autostainer Link 48 correlated with results obtained with Biocare Medical intelliPATH™ FLX. The following results also demonstrate that improved sensitivity and high intensity color development were achieved with detection reagents and chromogens from Enzo Life Sciences when compared to other detection reagents and chromogens from the competition.

MATERIALS

This study was designed to assess the sensitivity of POLYVIEW® nanopolymer detection reagents and the high intensity of the color development obtained with HIGHDEF® chromogens and their compatibility with automated slide stainers from Dako (Autostainer Link 48) and Biocare Medical (intelliPATH™ FLX). POLYVIEW® IHC reagent (mouse-HRP), POLYVIEW® IHC reagent (rabbit-HRP) and HIGHDEF® IHC chromogen substrate (DAB, stable) were compared to alternative products proposed by the competition (Table 1).

Reagent	Company	Catalogue number
POLYVIEW® mouse	Enzo Life Sciences	ADI-950-112
POLYVIEW® rabbit	Enzo Life Sciences	ADI-950-115
HIGHDEF® DAB	Enzo Life Sciences	ADI-950-212
EnVision™ mouse	Dako	K4001
EnVision™ rabbit	Dako	K4003
Liquid DAB+	Dako	K3468
ImmPress™ mouse	Vector Labs	MP-7402
ImmPress™ rabbit	Vector Labs	MP-7401
ImmPact™ DAB	Vector Labs	SK-4105

Table 1: List of detection reagents, chromogens and manufacturers

The sensitivity and the high definition of these reagents were evaluated using the same reagents for antigen retrieval, blocking, washes, primary antibody diluent and counterstaining (Table 2).

Reagent	Company	Catalogue number
Sodium Citrate Retrieval Buffer (100X) pH 6.0	Thermo	TA-250-PM1X
Tris Buffered Saline and Tween	Thermo	TA-999-TT
Peroxidase Block	Dako	S2023
Primary Antibody Diluent	Dako	S2022
Hematoxylin Z	CellPath	RBA-4201-00A

Table 2: List of IHC consumables

Comparison was done using four optimized antibodies against key cancer biomarkers used on a regular basis in batch-staining experiments within the Beatson Institute for Cancer Research: BrdU, Ki67 and p53 on formalin-fixed paraffin-embedded mouse intestinal tissue and pan-Cytokeratin on formalin-fixed paraffin-embedded human lung carcinoma tissue (Table 3).

Target	Antibody	Company	Catalogue number
BrdU	monoclonal (B44)	BD Biosciences	347580
pan-Cytokeratin	monoclonal (AE1/AE3)	Thermo	MS-343
Ki67	monoclonal (SP6)	Thermo	RM-9106
p53 (CM5)	polyclonal	Vector Labs	VP-P956

Table 3: List of targets, antibodies and manufacturers

METHODS

Tissue section pre-treatment

Consecutive tissue sections of 4µm thickness were prepared and left overnight in an oven set at 60°C. Sections were dewaxed in xylene for five minutes, rehydrated through 100% ethanol (2 x 1 minute) and 70% Ethanol (1 x 1 minute), and washed in deionized water for five minutes. Sections were retrieved using a solution of sodium citrate (1X) at pH 6.0 and a Dako Pre-treatment module for 25 minutes at 98°C. Sections were washed in Tris buffered Tween (TbT) before endogenous peroxidase activity was blocked using a peroxidase block for five minutes. Finally, tissue sections were washed in Tris buffered Tween (TbT).

Tissue section staining

Primary antibody was applied for 40 minutes at room temperature. Sections were washed twice in TbT before applying the detection reagents from Enzo Life Sciences, Dako or Vector Labs for 30 minutes. Sections were washed again twice. The chromogens from Enzo Life Sciences, Dako or Vector Labs were added and left on the tissue sections for 10 minutes. Sections were washed in deionized water for one minute before being counter-stained with hematoxylin Z for seven minutes. Sections were washed in deionized water for one minute, dipped twice in 1% acid alcohol and washed in deionized water for 30 seconds. Slides were then left for one minute in Scott's Tap Water Substitute and washed one more time in deionized water for one minute.

Tissue section post-staining

Slides were dehydrated with consecutive dips in 70% ethanol (1 x 1 minute), 100% ethanol (2 x 1 minute) and xylene (1 x 5 minutes). Tissue sections were then cleared and mounted with DPX mounting medium before being scanned using a fully automated large capacity Leica SCN400F slide scanner.

RESULTS

Immunohistochemistry on formalin-fixed paraffin-embedded mouse tissue

Using the POLYVIEW® and HIGHDEF® IHC reagents, BrdU, Ki67 and p53 were successfully stained on formalin-fixed paraffin-embedded mouse intestine tissue using a Dako Autostainer Link 48 (Fig. 1, images A-C). Staining also appeared more sensitive with residual background when compared to the staining obtained on subsequent sections with IHC reagents from the competition, notably for p53 with more p53-positive cells appearing with IHC reagents from Enzo Life Sciences (Fig. 1, images C, F and I). Similar results were obtained on Biocare Medical intelliPATH™ FLX (data not shown).

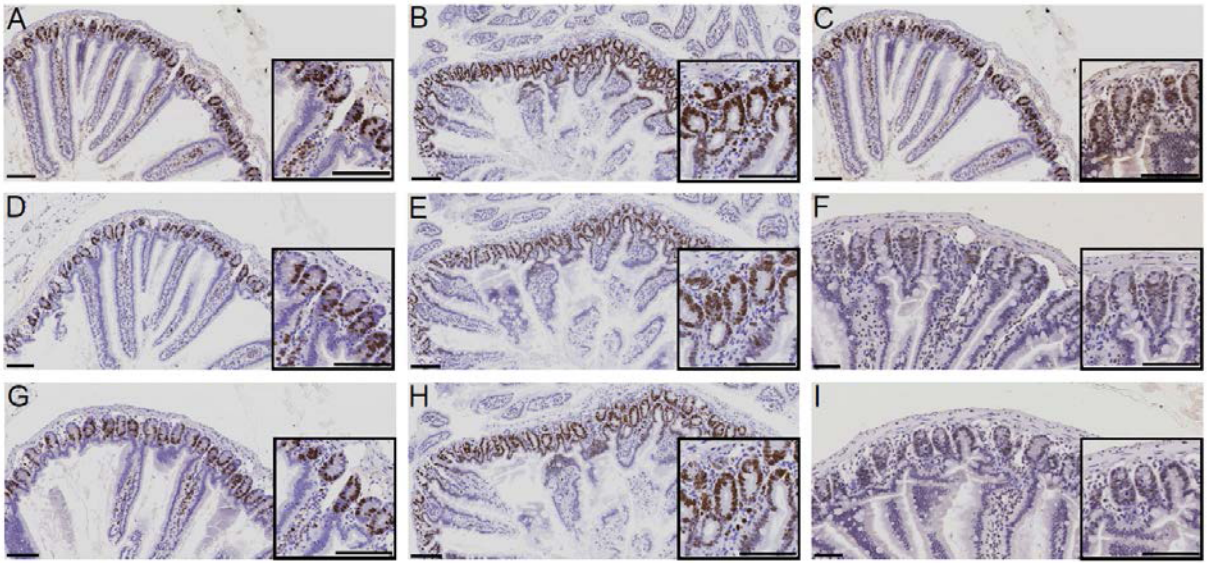

Figure 1: Expression of BrdU, Ki67 and p53 in mouse intestine. Immunohistochemistry staining of BrdU (A, D, G), Ki67 (B, E, H) and p53 (C, F, I) in formalin-fixed paraffin-embedded mouse intestine using detection reagents and chromogens from Enzo Life Sciences (A, B, C), Dako (D, E, F) and Vector Labs (G, H, I).

Immunohistochemistry on formalin-fixed paraffin-embedded human tissue

Using the POLYVIEW® and HIGHDEF® IHC reagents, pan-cytokeratin was successfully stained on formalin-fixed paraffin-embedded human lung carcinoma using a Dako Autostainer Link 48 (Fig. 2, image A). Staining also appeared more sensitive with residual background when compared to the staining obtained on subsequent sections with IHC reagents from the competition (Fig. 2, images B and C). Similar results were obtained on Biocare Medical intelliPATH™ FLX (data not shown).

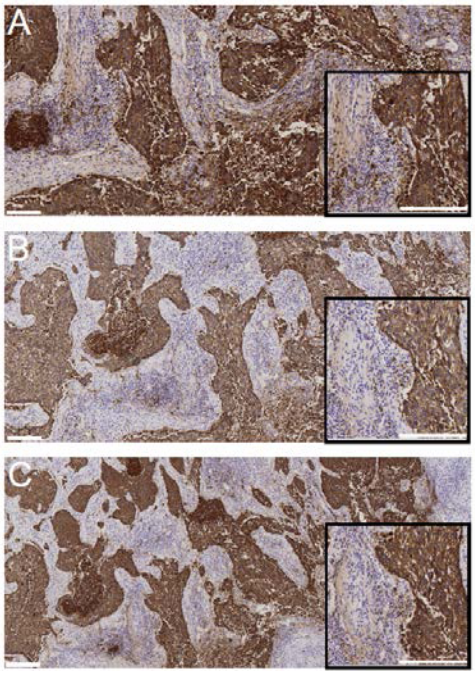

Figure 2: Expression of pan-cytokeratin in human lung carcinoma. Immunohistochemistry staining of Pan-cytokeratin in formalin-fixed paraffin-embedded human lung carcinoma using detection reagents and chromogens from Enzo Life Sciences (A), Dako (B) and Vector Labs (C).

CONCLUSION

The main objective of this work was to establish the compatibility of Enzo Life Sciences' novel IHC detection reagents and chromogens with two automated slide stainers and determine their potential in terms of sensitivity and intensity when compared with reagents offered by the competition. Application of the POLYVIEW® IHC detection reagent and HIGHDEF® IHC chromogen resulted in the successful detection of BrdU, Ki67 and p53 in murine intestine tissue and pan-cytokeratin in human lung tissue. The intensity of the staining was also maximized whilst minimizing residual background, thereby validating their use as ideal IHC reagents for automated staining regardless of the autostainer platforms.

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