Evaluation of Tissue Adhesion and Staining Performance of BOND Adhesive Microscope Slides: A Comparative Study on the BOND-III and BOND-MAX Platforms

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Abstract

Leica Biosystems, Richmond has developed the Apex BOND Adhesive Slide (Apex BOND slide) for use on the BOND platforms. The slides were tested in the BOND-III and BOND-MAX IHC instruments alongside the BOND Plus slides using a variety of tissues, antibodies, epitope retrieval methods, detection systems and oligonucleotide probes. This comparative study focused on performance of the Apex BOND slides and the BOND Plus slides when tested for tissue adhesion, specific staining and background chromogen pick-up on the glass. The Apex BOND slides performed as well as the BOND Plus slides in all criteria, and they are now available for use on the BOND platform.

Introduction

Immunohistochemical procedures require microscope slides that promote strong specific staining in the tissue specimen with minimal background staining on the glass slide. Tissue adhesion is also a critical requirement, as harsh pretreatment procedures and enzymatic digestion are known to cause tissue loss.

Leica Biosystems has redesigned the Apex BOND microscope slide for use on the BOND platform (Figure 1). The new Apex BOND slide, which is an expansion of the Apex portfolio of adhesive slides, has strategically placed markings on the glass surface to identify the usable areas for the two dispense volume settings on the BOND platform. The objective of the study was to test the Apex BOND slide for use on the BOND-III and BOND-MAX systems and compare its performance to the BOND Plus slide. Verified staining protocols for the BOND platform were performed using both the Apex BOND slides and BOND Plus slides. The results of each staining protocol were evaluated to determine if the Apex BOND slides performed as well as the BOND Plus slides.

Figure 1. The redesigned Apex BOND slide, available for use on the BOND platform.

Materials and Methods

A total of 900 Apex BOND (catalog number 3800040) and 900 BOND Plus (catalog number S21.2113.A) slides were tested, employing 5 BOND protocols, 10 antibodies, 2 oligonucleotide probes, 6 tissue types, 3 epitope retrieval methods and 2 detection methods (as outlined in Table 1 below). The testing scheme outlined in Table 1 was split evenly and run on both the BOND-III and BOND-MAX systems (450 Apex BOND and 450 BOND Plus on each system). Tissue sections were cut at three microns and placed on the Apex BOND and BOND Plus Slides. The slides were then dried in an oven at 60°C for one hour. The Apex BOND and BOND Plus slides were alternated on the slide trays for each run on each of the BOND-III and BOND-MAX systems. Each run consisted of a set of 30 slides (15 Apex BOND and 15 BOND Plus) that were loaded onto the three slide staining assemblies (SSAs) and stained with the same protocol and antibody or oligonucleotide probe in parallel.

Pretreatment methods were used to bring about a conformational change in the protein to expose the epitope in the tissue. The optimal pretreatment method is dependent upon several factors, including the antibody or oligonucleotide probe used for staining. Pretreatment methods include Heat Induced Epitope Retrieval (HIER) and Enzyme Induced Epitope Retrieval (EIER). Depending on the antibody or probe and detection system, either IHC Protocol F, IHC Protocol J, ISH Protocol A, Flippin Protocol*, or ISH Protocol B on both the BOND-III and BOND-Max instruments was selected. When using HIER, the slides with tissue were heated to 100°C. One of two epitope retrieval (ER) solutions, ER Solution 1 or ER Solution 2, was used with each antibody. ER Solution 1 (catalog number AR9961) consists of a citrate buffer with a pH of 5.9-6.1, while ER Solution 2 (catalog number AR9640) is an EDTA buffer with a pH of 8.9-9.1. The EIER, which heats slides to 37°C, was also used with some antibodies and the oligonucleotide probes. The Enzyme Pretreatment Kit (catalog number AR9551) consists of the Enzyme Concentrate, which contains a proteolytic enzyme and stabilizer, and the Enzyme Diluent, consisting of a Tris-buffered saline solution with a surfactant and 0.35% ProClin 950. One drop of Enzyme Concentrate was diluted in 7mL of Diluent to make Enzyme 1 used in these studies. Depending on the antibody used, the Enzyme 1 pretreatment was 5 or 10 minutes. The Enzyme 1 pretreatment for the ISH A Protocol was 15 minutes followed by a 2 hour hybridization step. The ISH B Protocol used a more diluted concentration of Enzyme 1 for 15 minutes along with ER 2 for 20 minutes followed by a 2 hour hybridization step.

Table 1. Testing Scheme

BOND Protocol	Tissue	Antibody Specificity	Antibody Clone	Detection System	Epitope Retrieval	Number of Slides - BOND-III	Number of Slides - BOND-MAX
IHC F	Bowel	S-100 BOND RTU Primary (PA0900)	Polyclonal	BOND Polymer Refine Detection	Enzyme	48	48
	Breast	Estrogen Receptor BOND RTU Primary (PA0151)	6F11	BOND Polymer Refine Detection	ER 1	44	44
	Breast	Progesterone Receptor BOND RTU Primary (PA0312)	16	BOND Polymer Refine Detection	ER 2	44	44
	Bowel	SMA BOND RTU Primary (PA0943)	alpha SM-1	BOND Polymer Refine Detection	None	44	44
IHC J	Melanoma	Melan A BOND RTU Primary (PA0233)	A103	BOND Polymer Refine Red Detection	ER 2	48	48
	Bowel	S-100 BOND RTU Primary (PA0900)	Polyclonal	BOND Polymer Refine Red Detection	Enzyme	44	44
	Melanoma	HMB45 BOND RTU Primary (PA0027)	HMB45	BOND Polymer Refine Red Detection	Enzyme	44	44
	Melanoma	Tyrosinase BOND RTU Primary (PA0322)	T311	BOND Polymer Refine Red Detection	ER 2	44	44
*Flippin	Tonsil	CD3 BOND RTU Primary (PA0553)	LN10	BOND Polymer Refine Detection	ER 2	48	48
	Bowel	CD34 BOND RTU Primary (PA0212)	QBEnd/10	BOND Polymer Refine Detection	ER 2	44	44
	Lung	TTF1 BOND RTU Primary (PA0364)	SPT24	BOND Polymer Refine Detection	ER 1	44	44
	Bowel	SMA BOND RTU Primary (PA0943)	alpha SM-1	BOND Polymer Refine Detection	None	44	44
ISH A	Tonsil	Novocastra Lambda Probe (ISH5770-A)	Analyte Specific Reagent	BOND Polymer Refine Detection	Enzyme and Hybridization	180	180
ISH B	Cervix - HPV+	Human Satellite DNA, Flu-labeled Probe (40V000V495)		BOND Polymer Refine Detection	Enzyme and Hybridization	180	180
					Total Slides	1800	

***Note: The Flippin Protocol is an amended protocol widely used by BOND customers.**

Tissue specimens were immunostained utilizing the antibodies, oligonucleotide probes, detection systems and protocols outlined above in Table 1. Following immunostaining, slides were coverslipped and examined. The slides were scored for each category according to the criteria outlined in the Results and Discussion section. Scores were averaged for each slide type (Apex BOND and BOND Plus), and the Apex BOND Slide average was compared to the average for the BOND Plus slides for each criteria: tissue adhesion, positive staining, and background chromogen on the glass.

Results and Discussion

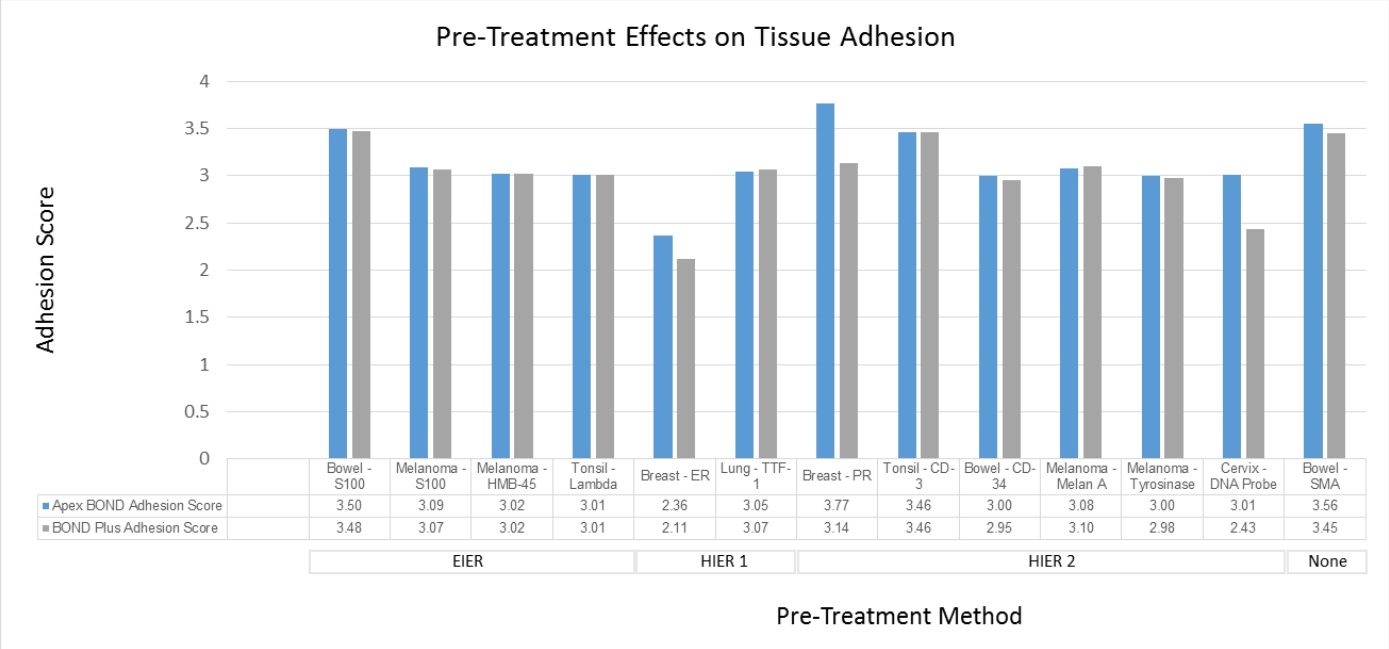
Tissue Adhesion

While factors such as poor fixation and processing can lead to tissue adhesion issues, a contributor of tissue loss during immunostaining is the harshness of pretreatment protocols used for epitope retrieval. These protocols include HIER as well as EIER. The effects of three pretreatments on tissue adhesion were evaluated. In the present study, no significant lifting or tissue loss resulted from either the IHC or ISH processes on the Apex BOND slides. Of all the tissues tested, only two significant differences between the Apex BOND slides and BOND Plus slides were observed. The Apex BOND slides provided better adhesion when testing breast tissue subjected to epitope retrieval method HIER with ER Solutions 1 and 2; and when testing HPV+ cervical tissue subjected to a combination of EIER and HIER with ER Solution 2 during ISH-B staining, as shown in Figure 2. The lifting of tissue from the Apex BOND and BOND Plus slide surfaces had no impact on the ability to read/grade the specific staining in the tissue.

Figure 2. Tissue Adhesion. Thirteen tissue and antibody combinations tested displayed varying amounts of lifting off the slides after processing on both the Apex BOND and BOND Plus slides. The lifting was predominantly edge lifting. Apex BOND outperformed the BOND Plus with adhesion of breast tissue for ER and PR, and cervical tissue for ISH-B DNA staining. HIER 1 refers to Heat-induced Epitope Retrieval using Epitope Retrieval Solution 1, and HIER 2 refers to Heat-induced Epitope Retrieval using Epitope Retrieval Solution 2, and EIER refers to Enzyme-induced Epitope Retrieval.

Adhesion

0 = Section Missing
1 = ~2/3 of Section Lifting
2 = ~1/3 of Section Lifting
3 = Edges of Section Lifting
4 = Entire Section Adhered

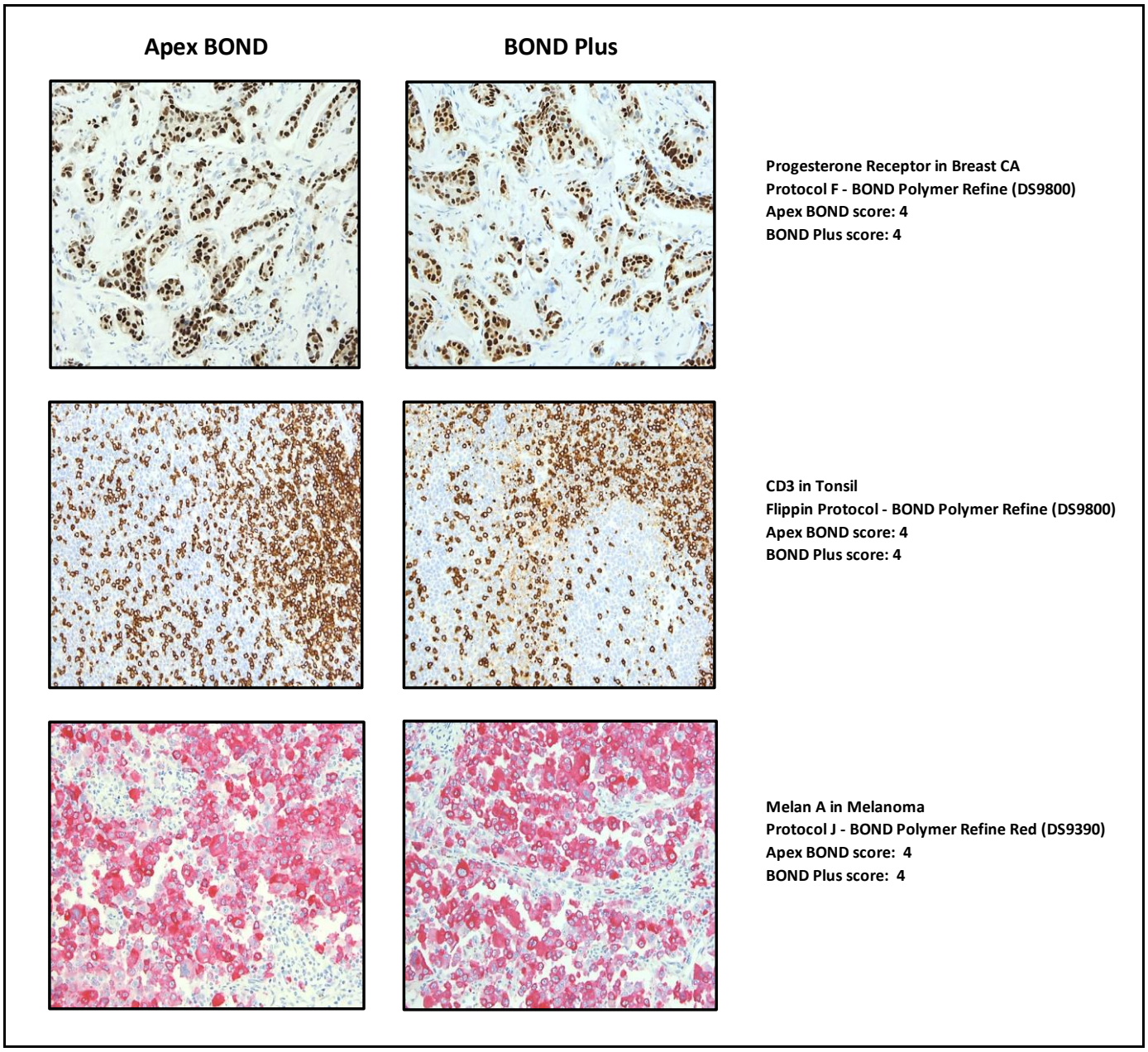


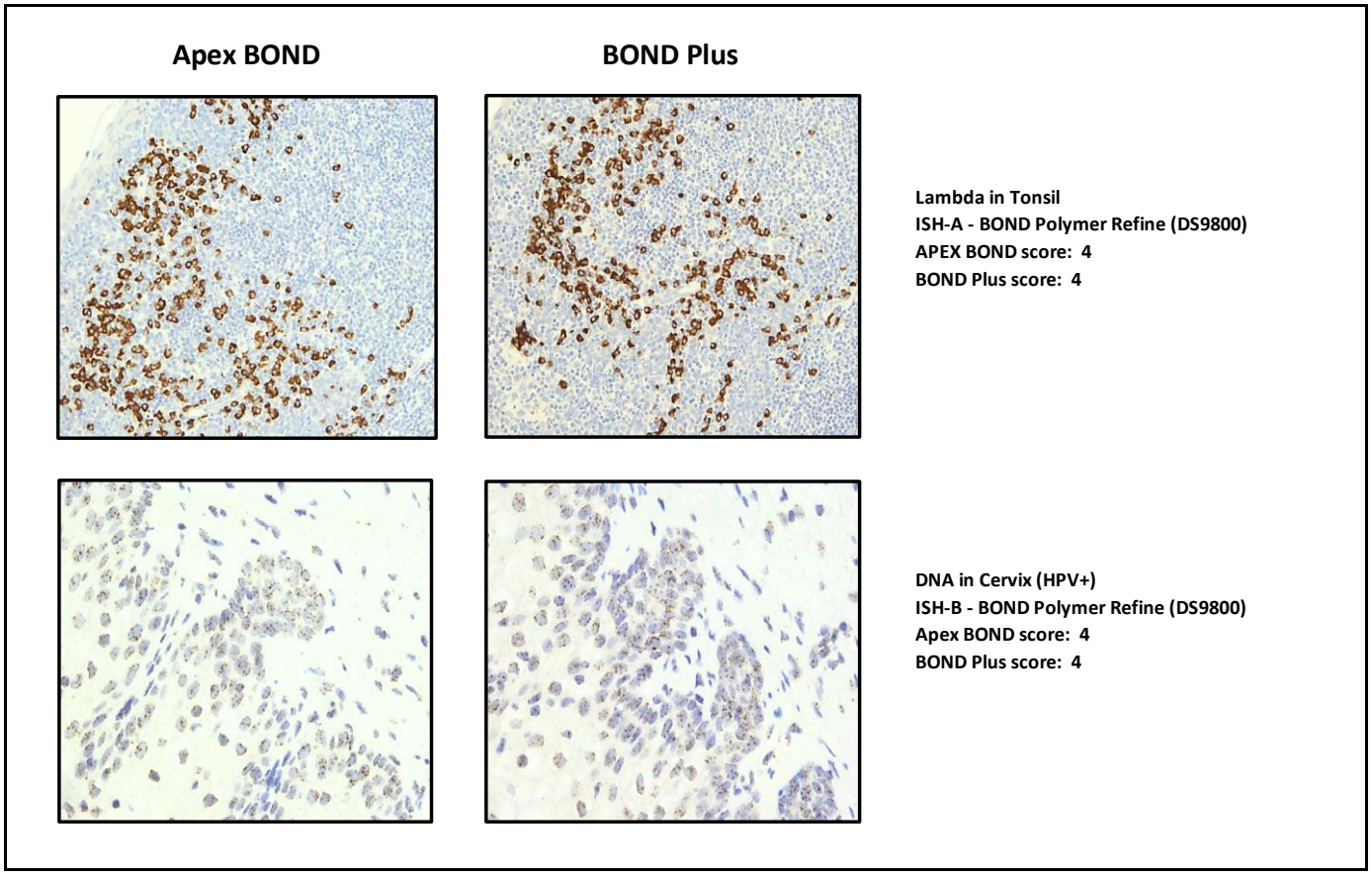
Specific Staining

Specimens stained on Apex BOND and BOND Plus slides demonstrated strong and specific immunoreactivity when stained with the antibodies or oligonucleotide probes, as displayed in Figure 3. Sixty runs (30 on BOND-III and 30 on BOND-MAX) were performed with 10 antibodies, 2 oligonucleotide probes, 6 tissue types, 3 epitope retrieval methods and 2 detection methods. No significant differences in the intensity of staining of specimens were observed on the Apex BOND versus the BOND Plus slides.

Figure 3. Specific Staining. Representative examples of specific staining in several tissues using five different protocols, and their respective antibody/probe, epitope retrieval methods and detection systems are shown.

Specific Staining
0 = Little or no immunostaining
1 = Weak immunostaining
2 = Acceptable immunostaining
3 = Good immunostaining
4 = Excellent immunostaining

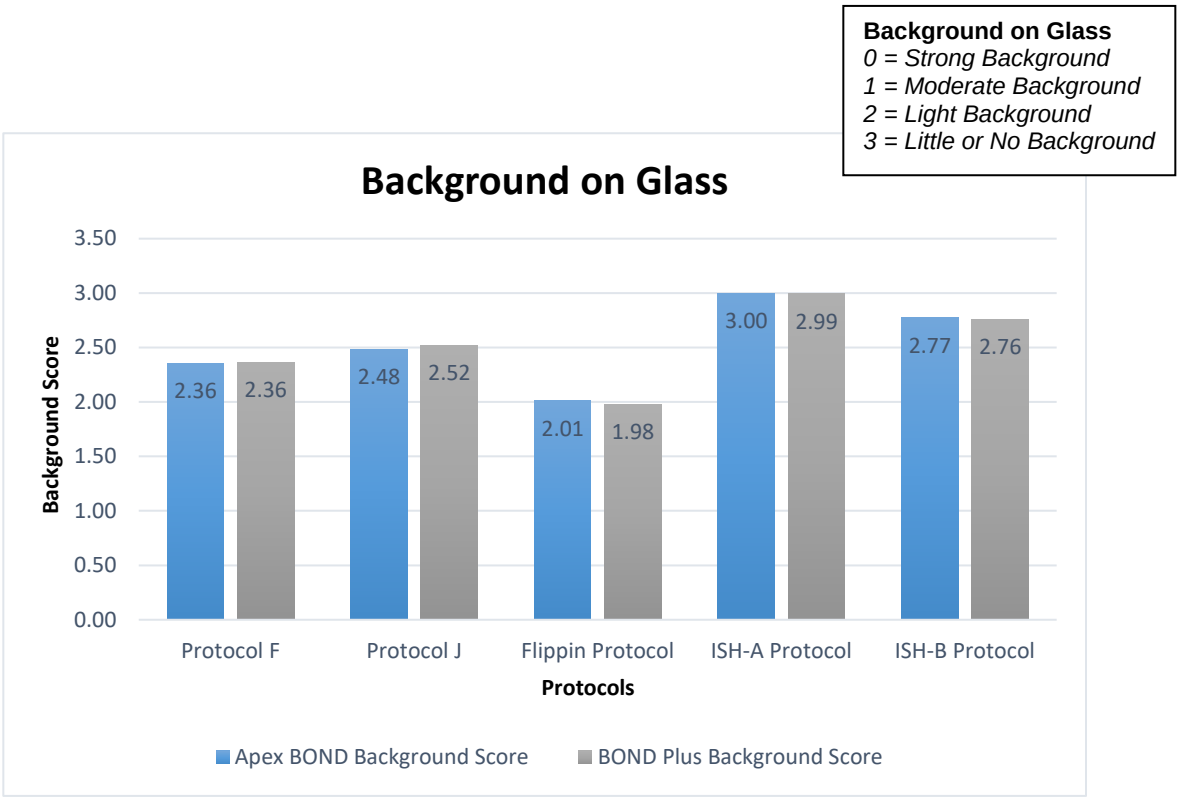




Background Chromogen Staining

Background staining on areas of the glass slide not occupied by the tissue specimen may occur due to nonspecific attachment of one or more of the components of the primary antibody, oligonucleotide probe, or detection system. Background staining on areas of the glass slide not occupied by the tissue was minimal or absent on the Apex BOND slides when specimens were stained according to the testing scheme in Table 1. No significant differences were observed between the background scores on the Apex BOND versus the BOND Plus slides. The results of the scoring for background staining are represented in Figure 4. Scoring for background staining also factored any microscopic background observed within the tissue sections. The table in Figure 4 demonstrates no significant differences between microscopic background observed within the same tissue sections stained on each slide type (Apex BOND vs. BOND Plus).

Figure 4. Mean background scoring. Mean scores of five protocols run on both BOND-III and BOND-MAX displayed varying amounts of background staining, as depicted in the graph. Background staining was scored based on chromogen present both on the glass areas around the tissue and microscopically within the tissue section.



Conclusions

The newly redesigned Apex BOND slides were tested in both the BOND-III and BOND-MAX IHC instruments alongside the BOND Plus slides using a variety of tissue types, antibodies, epitope retrieval methods, detection systems and two oligonucleotide probes. This comparative study demonstrated that the Apex BOND slides performed as well as the BOND Plus slides when tested for tissue adhesion, specific staining and background chromogen pick-up on the glass. The previous validation done for Apex BOND slides tested 510 slides and employed 10 antibodies, 1 oligonucleotide probe for ISH testing, 8 tissue types, 3 epitope retrieval methods and 2 detection methods (data not shown). Again, the Apex BOND slides performed as well as the BOND Plus slides when tested for the same criteria. Therefore, these studies demonstrate that the Apex BOND slides perform as well as the BOND Plus slides on the BOND-III and BOND-MAX instruments, and demonstrates that the Apex BOND slides can be used on these BOND platforms.

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