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

By Michelle Stemler, Stanley E. Hansen, Megan Heron, Jay Mohanarajulu, Lise W. Duran, and Russell Myers

Abstract

Leica Biosystems, Richmond has developed the Apex BOND Adhesive Slide (Apex BOND) slide for use on the BOND platform. The slides were tested in the BOND-III IHC instrument alongside the Leica BOND Plus (BOND Plus) slides using a variety of tissues, antibodies, epitope retrieval methods, detection systems and an oligonucleotide probe. 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 designed a new microscope slide for use on the BOND platforms (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 platforms. The objective of the study was to test the Apex BOND slide for use on the BOND-III system and compare its performance to the BOND Plus slide. Experiments were performed using both the Apex BOND slides and BOND Plus slides. The results of each experiment were evaluated to determine if the Apex BOND slides performed as well as the BOND Plus slides.

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

Materials and Methods

A total of 255 Apex BOND (catalog number 3800040) and 255 BOND Plus (catalog number S21.2113.A) slides were tested, employing 13 antibodies, 1 oligonucleotide probe, 8 tissues types, 3 epitope retrieval methods and 2 detection methods (as outlined in Table 1 below). 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 BOND-III slide trays for each run. One 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, or *ISH Protocol A on the BOND-III instrument (material number 21.2201) was selected. When using

HIER, the slides with tissue were heated to 100°C. One of two epitope retrieval (ER) solutions, ER Solution I or ER Solution II, was used with each antibody. ER Solution I (catalog number AR9961) consists of a citrate buffer with a pH of 5.9-6.1, while ER Solution II (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 probe. 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 was 15 minutes followed by a 2 hour hybridization step.

Table 1. Testing Scheme

Epitope Retrieval	Tissue	Antibody Specificity	Antibody Clone	Detection System	BOND-III Protocol	Number of Slides
ER I	Breast	Estrogen Receptor	6F11	BOND Polymer Refine Detection	*IHC F	15
	Tonsil	CD20	MJ1	BOND Polymer Refine Detection	*IHC F	15
	Appendix	CD20	MJ1	BOND Polymer Refine Detection	*IHC F	15
	Skin	Vimentin	V9	BOND Polymer Refine Detection	*IHC F	15
	Brain	Neurofilament 200kD	N52.1.7	BOND Polymer Refine Detection	*IHC F	15
	Intestine	Chromogranin A	5H7	BOND Polymer Refine Detection	*IHC F	15
ER II	Uterus	Progesterone Receptor	16	BOND Polymer Refine Detection	*IHC F	15
	Tonsil	CD3	LN10	BOND Polymer Refine Detection	*IHC F	15
	Appendix	CD3	LN10	BOND Polymer Refine Detection	*IHC F	15
	Skin	Desmin	DE-R-11	BOND Polymer Refine Detection	*IHC F	15
	Skin	Melan A	A103	BOND Polymer Refine Red Detection	*IHC J	15
Enzyme	Prostate	High Molecular Weight Multi-Cytokeratin	34βE12	BOND Polymer Refine Detection	*IHC F	15
	Skin	High Molecular Weight Multi-Cytokeratin	34βE12	BOND Polymer Refine Detection	*IHC F	15
	Tonsil	Kappa Oligonucleotide Probe	N/A	BOND Polymer Refine Detection	*ISH A	15
	Skin	Multi-Cytokeratin (AE1/AE3)	AE1 and AE3	BOND Polymer Refine Detection	*IHC F	15
	Skin	S-100	Polyclonal	BOND Polymer Refine Detection	*IHC F	15
No Pre-Treatment	Prostate	Prostatic Acid Phosphatase	PASE/4LJ	BOND Polymer Refine Red Detection	*IHC J	15

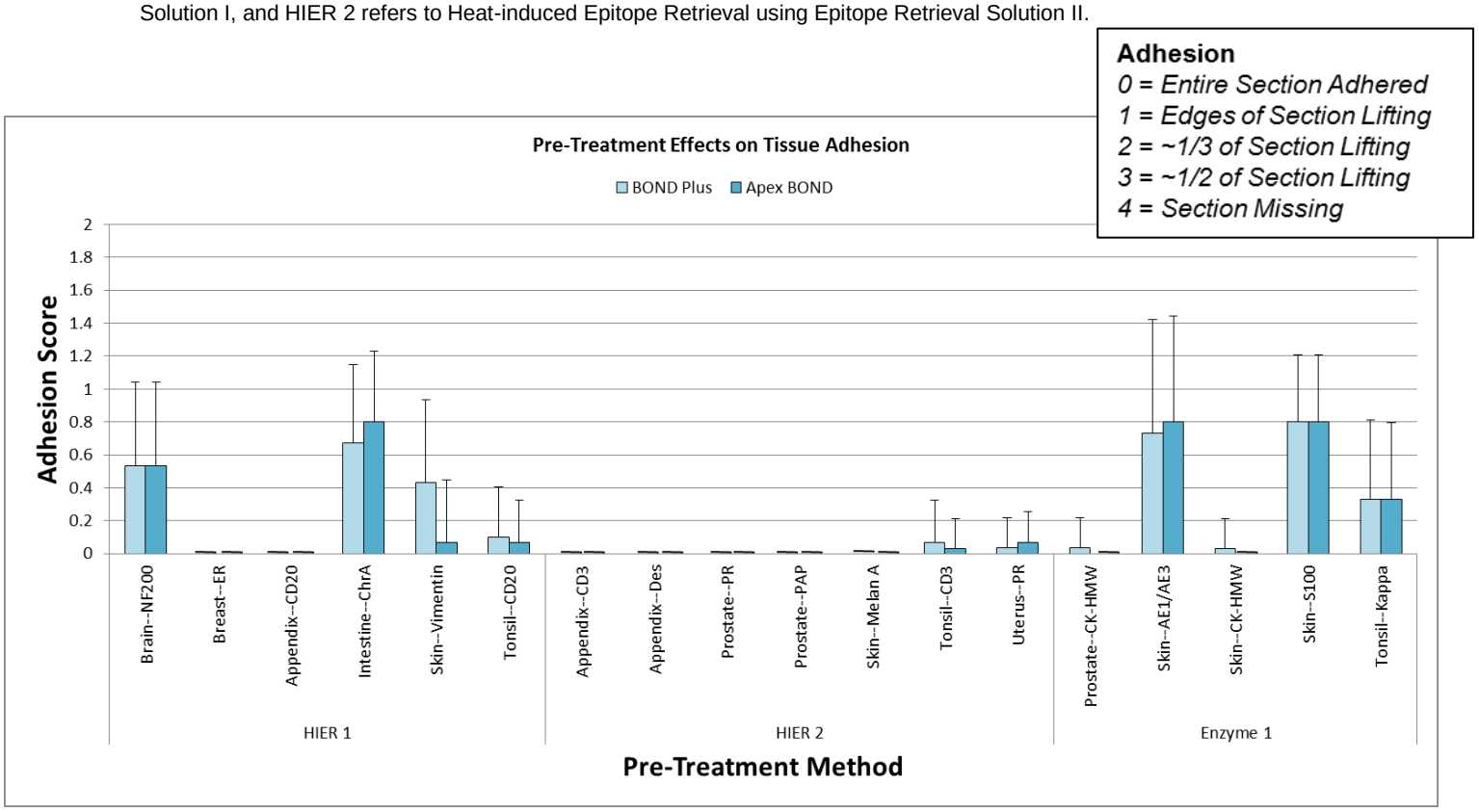
Tissue specimens were immunostained utilizing the antibodies, oligonucleotide probe, detection systems and protocols outlined above in Table 1. Following immunostaining, slides were coverslipped and examined. The slides were scored for each category according to the criteria outlined in the Results and 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 pick-up on the glass. A two-tailed T-test was used to evaluate differences between the two slide types. A T-test result of $p \leq 0.05$ indicated a significant difference.

Results and Discussion

Tissue Adhesion

A major contributor of tissue loss during immunostaining is the 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 notable lifting and no tissue loss resulted from either the IHC or ISH processes. Several tissue types that are known to be particularly susceptible to detachment during pretreatment (breast, brain, skin) remained adhered to the slides. Of all the tissues tested, only one significant difference between the Apex BOND slides and BOND Plus slides was observed. The Apex BOND slides provided better adhesion when testing skin tissue subjected to epitope retrieval method HIER with ER Solution I, as shown in Figure 2. However, in this case, the lifting of tissue from the BOND Plus surface was confined to the edges of the tissue, and therefore, the lifting had no impact on the ability to read/grade the specific staining in the tissue.

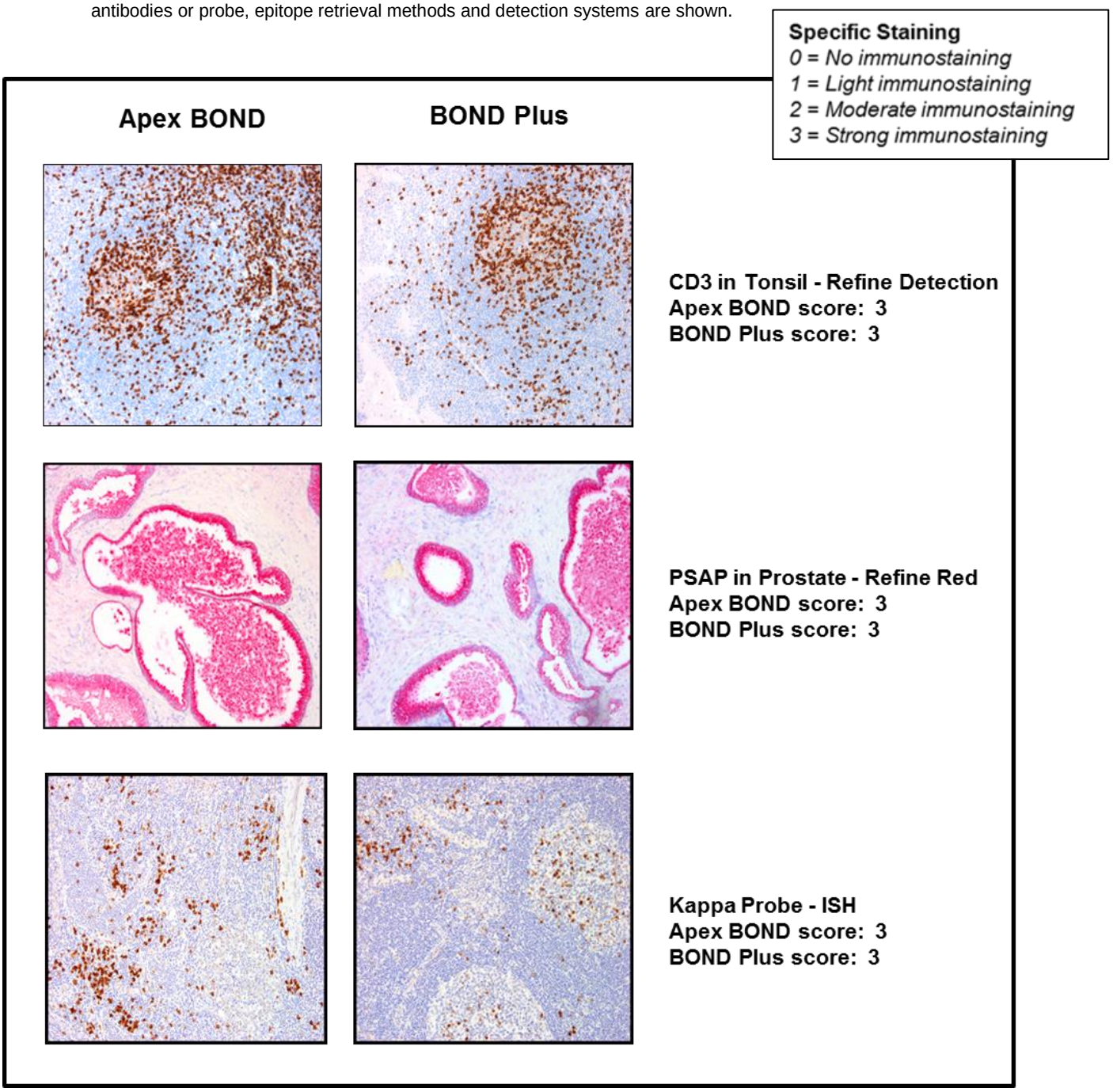
Figure 2. Tissue Adhesion. Eleven tissues tested displayed lifting off the slides after processing on either the Apex BOND or BOND Plus slides. The other seven tissues tested showed no lifting after processing. Error bars represent the standard deviation among the slides in the run. HIER 1 refers to Heat-induced Epitope Retrieval using Epitope Retrieval Solution I, and HIER 2 refers to Heat-induced Epitope Retrieval using Epitope Retrieval Solution II.



Specific Staining

Specimens stained on Apex BOND and BOND Plus slides demonstrated strong and specific immunoreactivity when stained with the antibodies or an oligonucleotide probe, as displayed in Figure 3. Seventeen runs were performed with 13 antibodies, 1 oligonucleotide probe, 8 tissues 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 different antibodies or probe, epitope retrieval methods and detection systems are shown.



Background Chromogen Staining

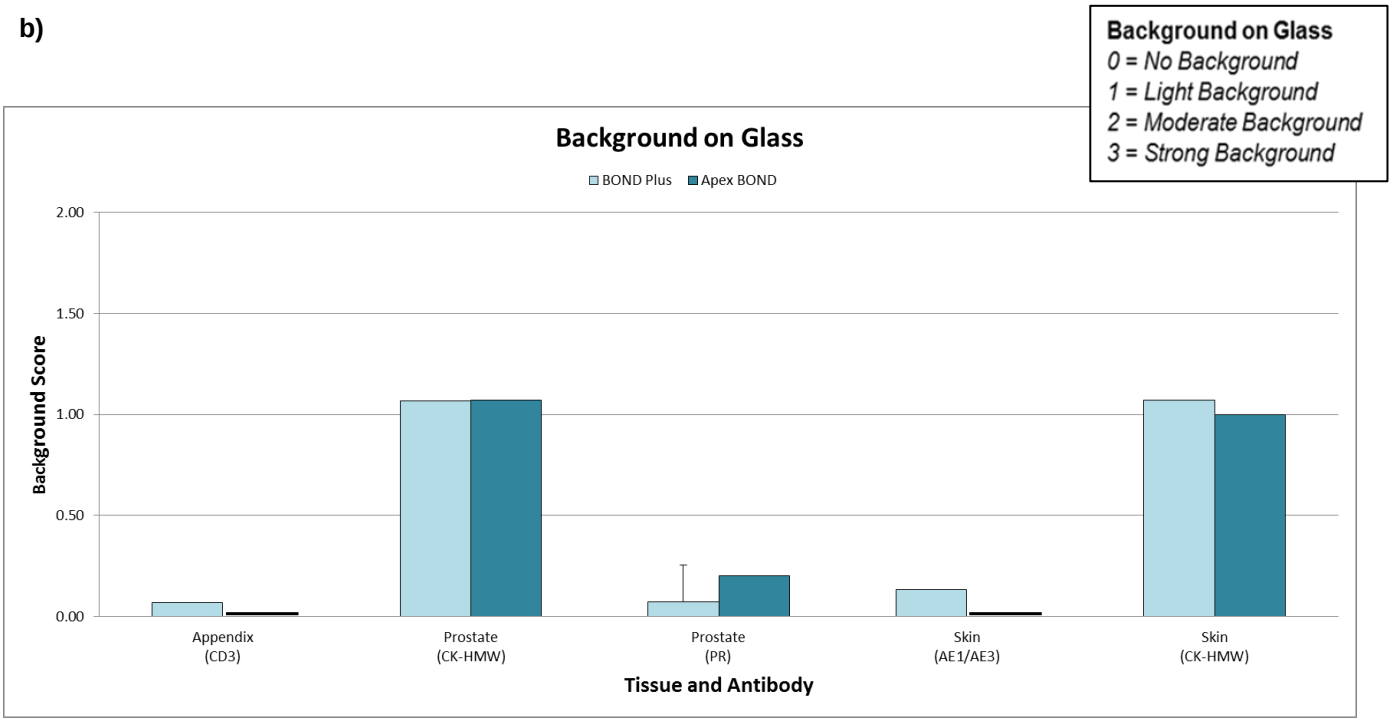
Background staining on areas of the glass slide not occupied by the tissue specimen may occur due to nonspecific attachment of one or more of the components of the primary antibody, oligonucleotide probe, or detection system. Background staining was minimal or absent on the Apex BOND slides when specimens 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.

Figure 4. Background chromogen staining on glass slides. a) Prostate tissue stained with high-molecular weight cytokeratin (CKHMW) antibody. The two negative slides on the right, one Apex BOND and one BOND Plus slide, did not receive the CKHMW antibody. b) Five tissues/antibodies displayed background staining, as depicted in the graph (error bars represent the standard deviation of samples in the run). No background staining was observed with the other twelve tissues/antibodies or probe.

a)

BOND Plus + CKHMW | **Apex BOND + CKHMW** | **BOND Plus Control** | **Apex BOND Control**

b)



Conclusions

The newly developed Apex BOND slides were tested in the BOND-III IHC instrument alongside the BOND Plus slides using a variety of tissues, antibodies, epitope retrieval methods, detection systems and an oligonucleotide probe. 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. A separate study was also performed, testing 135 slides and employing 8 antibodies, 1 oligonucleotide probe for ISH testing, 5 tissues, 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 suggest the Apex BOND slides can be used on all BOND platforms.

Contributors

Michelle Stemler, M.S.
Project Lead, R&D
Leica Biosystems, Richmond

Stanley E. Hansen, M.A.
Research Scientist, R&D
Leica Biosystems, Richmond

Megan Heron
Program Manager, R&D
Leica Biosystems, Richmond

Jay Mohanarajulu, M.S.
Global Product Manager, Product Management, CHC
Leica Biosystems, Richmond

Lise W. Duran, Ph.D.
Vice President, R&D
Leica Biosystems, Richmond

Russell Myers, Ph.D.
Director, R&D
Leica Biosystems, Richmond