

## Abstract

Using conventional Immunohistochemistry protocols, large tissue sections of colorectal tumors or lymphoid tissues may produce patchy or incomplete staining of tissues. Since diffusion is the only mechanism by which antibodies reach their tissue targets, we examined whether immunostains may be improved by slide agitation of the overlying antibody and reagent solutions. The SlideBooster™ SB1800 Incubation Station of Advantix AG (Brunnthal, Germany) used in this study gently and mechanically agitates the antibody solution through surface acoustic wave (SAW)

technology thereby significantly improving penetration of antibodies into tissues. In addition, results with several antibody markers yielded a significant improvement in both contrast and signal intensity, and also showed an increase in the size of the stained tissue surface area from 15% to 75%. There was no destruction of tissue and cell morphology found.

## Introduction

The SlideBooster™ SB1800 Incubation Station of Advantix AG (Brunnthal, Germany) used in this study (Figures 1 & 2) agitates the overlying antibody solution through surface acoustic waves (SAWs) that are generated by interdigitating electrodes on transducer piezoelectric chips which are embedded in the instrument floor. The SAWs travel through the slide and tissue thereby mechanically and gently agitating the antibody solution by acoustic streaming (Figure 3). Simply stated, SAWs may be viewed as "miniature earthquakes" that more effectively agitate the antibody solution overlying the tissue section. Unlike conventional ultrasound in which certain frequencies have been known to damage tissue samples, SAW technology consists of much higher frequency (> 100 MHz) and of smaller amplitude (<1nm).

The SlideBooster™ SB1800 Incubation Station has three incubation chambers that allow the incubation of a total of 18 slides per unit. In addition, by employing an intuitive user interface, one PC can control up to 4 SlideBooster™ SB1800 instruments.



Figure 1.

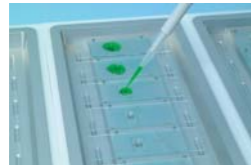


Figure 2.

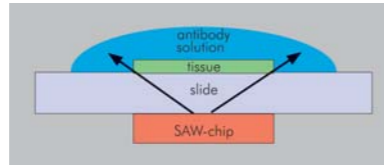


Figure 3.

## Purpose

The purpose of this study is to demonstrate the effects of mechanical agitation of the antibody solution using SAW technology on the quality of immunohistochemically stained tissue sections. There are numerous studies in the literature evaluating the effects of heat-induced epitope retrieval techniques and it was of interest to see the results of an alternative method in trying to improve staining quality. Notably, uneven

formalin fixation or poor diffusion of antibody reagent on tissue slides commonly results in patchy immunohistochemical staining. This study attempts to assess the staining improvement in large tissue sections (size > 1 cm) that have been formalin-fixed and paraffin embedded.

## Materials and Methods

Two identical sets of tissue slides were set up to run using two different manual protocols in parallel. One set of slides was run on a horizontal platform using our manual procedure in a humidified staining chamber. Concurrently, we also ran an identical second set of tissue slides using the SlideBooster™ SB1800 Incubation Station following the manufacturer's instructions. It should be noted that the SlideBooster™ SB1800 was used for all incubation steps—except during the chromogen (DAB) procedure. A variety of tissue samples were also evaluated including normal tonsils, colorectal carcinomas, melanoma, and other carcinomas.

Histologic sections of 4-5 µm were cut from paraffin-embedded tissue blocks, mounted on positively-charged glass slides, baked, deparaffinized, cleared in xylene and alcohol, and subsequently were rehydrated to water. Endogenous peroxidase was quenched by immersing the tissue sections for 10 minutes in 3% hydrogen peroxide in distilled water. As listed in Table 1, standard antigen retrieval techniques were performed using low & high pH Target Retrieval Solutions (DakoCytomation Carpinteria, CA). The tissue sections were first incubated using the primary antibody for 60 minutes at ambient temperature. The detection system consisted of a 25-minute incubation step (with post-blocking a Polymer Penetration Enhancer). This was followed by a second 30-minute incubation step using a secondary antibody consisting of a Poly-HRP anti-Mouse/Rabbit IgG. Both these antibody reagents were obtained from a PowerVision™ Histostaining Kit (Immunovision Technologies, Daly City, CA). The chromogen of choice was Diaminobenzidine (DAB). Finally, the slides were counterstained with hematoxylin.

Antibody	Antigen	Conc. (µg/ml)	Incubation Time (min)	SAW Intensity
BCL-6	Tonsil	1:1000	60	0
CD 3m	Tonsil	1:1000	60	+0.5
WT-1	Ovarian Tumor	1:1000	60	+0.5
CK 5/6	Carcinoma	1:1000	60	+1
hMLH1	Colo. Ca.	1:1000	60	+1
S100	Melanoma	1:1000	60	+1
TTF-1	Lung Carcinoma	1:1000	60	+1
VIMENTIN	Tonsil	1:1000	60	+1
CD 20	Tonsil	1:1000	60	+2
TS	Colo. Ca.	1:1000	60	+2

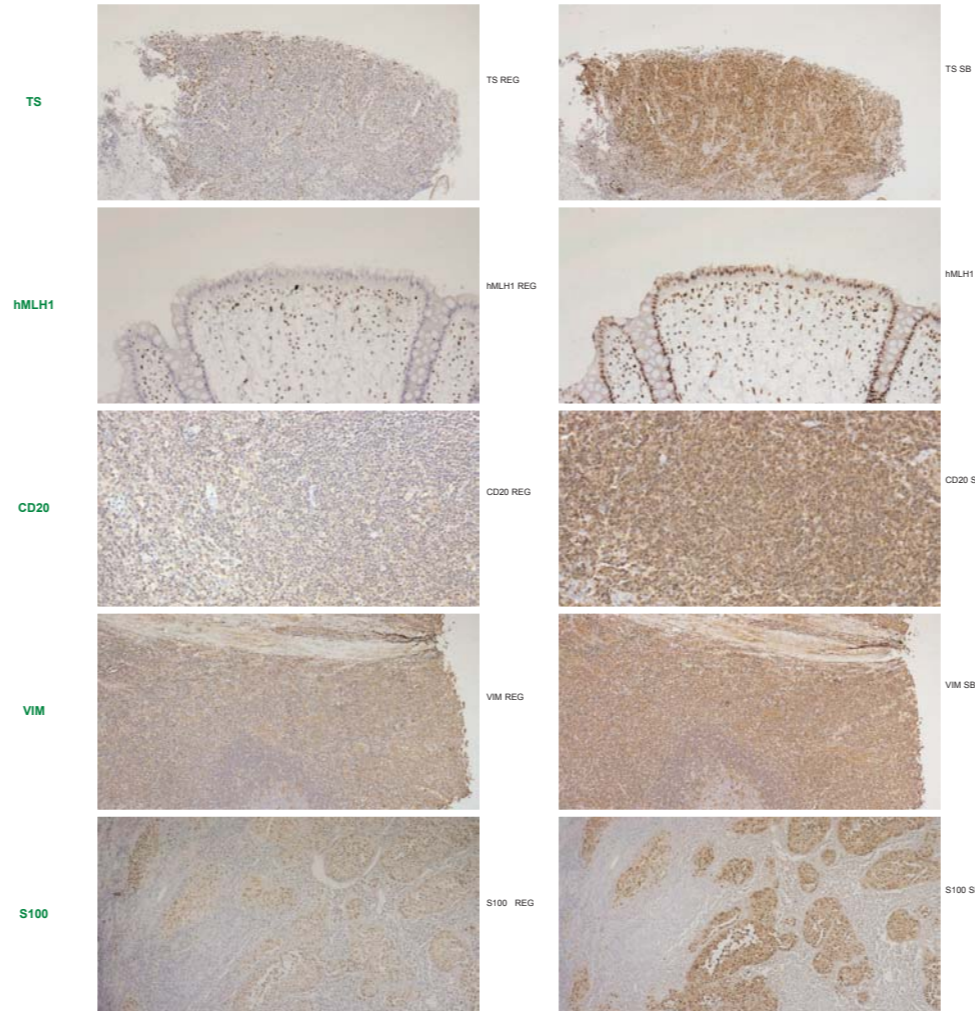
Table 1. Antibody list, titers and antigen retrieval procedure

## Results

### Antibody

### Without SlideBooster™

### With SlideBooster™



Antibodies Tested	Tissues Used	Relative Improvement of Staining Intensity
BCL-6	Tonsil	0
CD 3m	Tonsil	+0.5
WT-1	Ovarian Tumor	+0.5
CK 5/6	Carcinoma	+1
hMLH1	Colo. Ca.	+1
S100	Melanoma	+1
TTF-1	Lung Carcinoma	+1
VIMENTIN	Tonsil	+1
CD 20	Tonsil	+2
TS	Colo. Ca.	+2

Staining Intensity
0 = No Staining
1 = Weak (Slight)
2 = Moderate (Significant)
3 = Strong (Marked)

Table 3: \* Staining Intensity Scale:

Table 2 demonstrates that among the 10 antibodies tested, the SlideBooster™ improved immunohistochemical staining intensity of 9 antibodies. Staining intensity of the tissues was graded on a 0, 1, 2 or 3+ scale\*. The relative improvement was recorded as an increase in staining intensity. Table 3 describes the staining intensity of histologic sections.

The results range from no improvement in BCL-6, to slight improvement in CD3m, and WT-1, to significant improvement in CK 5/6, CD20, BCL-6, and WT-1. The results of the diluted antibodies were more dramatic since the SlideBooster™ slides demonstrated a much higher relative staining intensity than the non-SlideBooster™ stained slides. However, it should be noted that there was no improvement for BCL-6—which may be due to an inherent characteristic of the antibody itself. In addition, using the TS and hMLH1 antibodies, we observed an increase of the size of the stained area from 15%-75% with significant improvement in both contrast and signal intensity.

Finally, another finding that was noted involved the size of the tissue area that was stained. Since the version of the SlideBooster™ SB1800 Incubation Station used in this study was equipped with only one mixer chip per slide, the effective slide tissue area agitated by each mixer chip is approximately 22 mm in greatest diameter. Therefore, tissues that exceeded this diameter did not show improvements outside this active area. The manufacturer is fully aware of this and future releases of this instrument will accommodate larger tissues with multiple transducers per slide.

## Conclusions

Analysis of the results reveal significant to marked improvement in most of the antibodies tested without compromising sensitivity, quality of staining and tissue morphology. Notably, the SlideBooster™ allows for increased antibody dilution and logically, will enable cost savings of reagents. Because of the improved staining, a decreased repeat rate would also be expected. In fact, instead of diluting the antibodies, turn-around time (TAT) may be improved by shortening the incubation time of the assay. It may be worthwhile to investigate this aspect in a future study.

## References

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