Assessing Adhesion Slide Performance Across Histology Applications

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OBJECTIVES

- Analyze the differences in contact angles and in tissue adherence during microtomy
- Investigate whether different adhesion slides exhibit similar levels of background staining during histological staining procedures
- Evaluate and compare the tissue adhesion properties of adhesion slide brands across different tissue types and applications

BACKGROUND

Adhesion slides are widely preferred for IHC to aid in securing tissue sections to the slide and prevent reworks that could potentially postpone a patient diagnosis and drive-up costs in the lab. The cost of reworking a failed IHC slide due to poor tissue adhesion is estimated to be ~$80 per slide, considering the reagent cost and workload administration.[1] Adhesion slides reinforce tissue adherence and integrity, minimizing the need to recut and restain the sample to ensure proper tissue morphological characteristics. Adhesion slides may also be used for H&E stains and special stains for added adhesion, but could retain excess reagent, or background staining, on the slide.

MATERIALS AND METHODS

Slides
- StatLab Millennia™ 1000 (M1000)
- StatLab Millennia™ Command (MCOMM)
- StatLab Millennia™ 2000 (M2000)
- StatLab InkPro™ +
- StatLab KT3+™
- StatLab KT5+™
- StatLab Colorview™ +
- Marienfeld HistoBond® S+
- Matsunami TOMO®
- DAKO Flex
- Epredia SuperFrost™ +

Stains
- StatLab Vintage Hematoxylin
- StatLab Reserve Hematoxylin
- StatLab Gill 3 Hematoxylin
- Quantum HDx Antigen Retrieval kits
- MasterTech GMS Stain Kit

Instruments
- Biochrom Libra UV-visible Spectrophotometer
- Quantum HDx IHC Stainer
- MYR SS-30 Stainer
- KRUSS Drop Shape Analyzer DSA100E

Contact Angle
The measurement of water droplet dispersion onto the slide surface is also used to determine the hydrophilicity / hydrophobicity of a slide's surface chemistry[2]. A KRUSS Drop Shape Analyzer was utilized to measure 1mm of distilled water onto 8 locations on the slide and analyze the contact angle of the water as it met the slide's surface (see Table 2).

Water Bath Behavior
When picking up tissue sections in a water bath, tissue can "jump" onto the slide (hydrophobic) or the slide "chases" the tissue prior to picking up leaving a thin layer of water spread underneath which allows the section to be positioned (hydrophilic). A "hybrid" slide exhibits dual behaviors: the section quickly jumps onto the slide but the tissue does not anchor completely, allowing the tissue to be re-positioned. Three slides of each type were used to pick up different tissues and observed if the tissue "jumped", "chased" or exhibited both behaviors. Placenta, lung and breast tissues were sectioned, placed in a waterbath and 3 histotechs were observed using their preferred method of picking up sections: using forceps to attach tissue to slide or using only the slide to pick up sections. Behavior of each slide was documented (see Table 2).

H&E Testing
H&E staining was performed on all slides to determine reagent coverage, adhesion and any excess stain remaining with spectrophotometer measurements. 21 slides of each brand/type were stained and assessed for tissue adhesion and reagent coverage. Samples of gut and fat were sectioned on each slide at 4 microns, incubated/dried, and stained on a Myr SS-30 automated stainer with three different hematoxylins: StatLab Vintage, StatLab Reserve, and StatLab Gill 3. Slides were assessed visually for tissue adhesion, tissue adhesion, and reagent coverage. (see Tables 1 and 2).

Spectrophotometer Testing Protocol
Spectrophotometer testing was performed using the Biochrom Libra UV-visible Spectrophotometer to measure how much background staining remained on each slide post-staining. A tissue-free slide of each slide brand/type was run through the spectrophotometer first as a reference, followed by the H&E stained slide of the same type. This testing was done to compare the intensity of any background color (see Tables 1 and 2).

Special Stains Testing
Grocott Methenamine Silver (GMS) is a high-volume silver stain notorious for background staining. A GMS special stain was completed on each slide to assess background staining. Positive tissue for GMS was sectioned onto each slide at 4 microns and stained with a GMS stain kit using the manufacturer's suggested protocols. Following testing, slides were examined visually for background staining (see Tables 1 and 2).

IHC Testing
Tissue adhesion is one of the most important factors in Immunohistochemical (IHC) staining due to the aggressive nature of antigen retrieval. IHC staining was performed on each adhesion slide to assess adhesion using a tissue microarray block composed of easy difficulty tissues (lymph, appendix, spleen, kidney), medium difficulty tissues (lung, foreskin, placenta, cervix, melanoma, colon), and hard difficulty tissues (skin, fat, breast) sectioned onto slides at 4 microns, and dried for 50 minutes at 65°C. Tissue difficulty is based on the expectation of tissue wash or detachment based on combined knowledge in the field of histology. Appendix and spleen very rarely become detached where breast is well known to have tissue wash[3]. Antigen retrieval solutions at pH 6, pH 8, and pH 9 were used to include standard options available and to assess the aggressiveness of each one. After staining, each tissue section was graded microscopically for tissue adhesion (see Tables 1, 2, 3 and 4).

RESULTS

The differentiator for adhesion slides was apparent with IHC tissue adhesion. Easier tissues such as appendix and kidney performed well with most slides showing minimal failures. Variations in adhesion performance become more noticeable with medium difficulty tissues but was most substantial with tissue with hard difficulty, like breast. Failure rates due to tissue wash, folding, and separation with more difficult tissue were observed at a rate over 50% in more than half of the slide types tested (see Tables 3 and 4). This may result in additional material costs and histotech workload. Background staining showed some variation for both H&E and Special Stains. While excessive slide background may not affect tissue staining, it could be cause for an unacceptable slide for digital pathology and/or pathologist review, resulting in extra time and costs to repeat the stain. While contact angle and waterbath behavior affects tech workflow and preference, data did not support a correlation between slides for higher adhesion and lower background staining.

Chart 1 Adhesion and Background Staining Results for All Adhesion Slides Tested

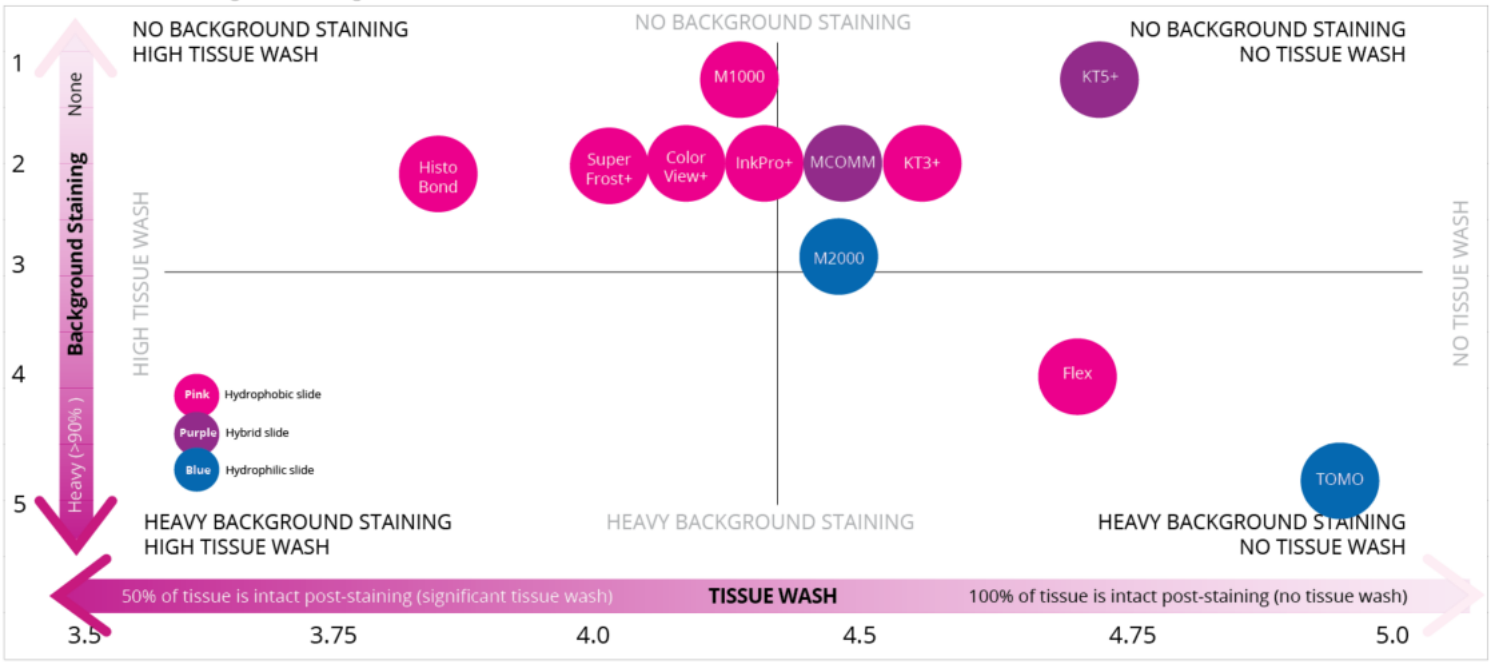

Image 1 Special stain testing (GMS) assessing background staining

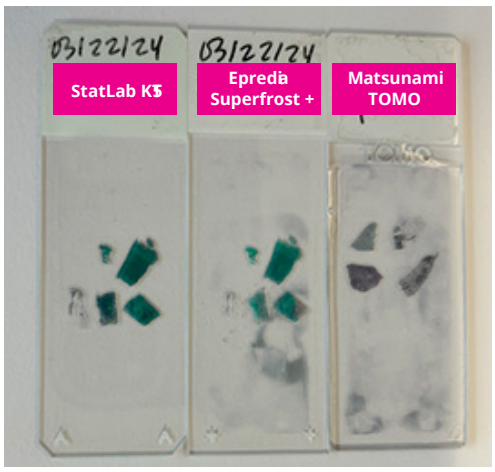

Image 2 Adhesion of breast tissue after IHC (pH 6)

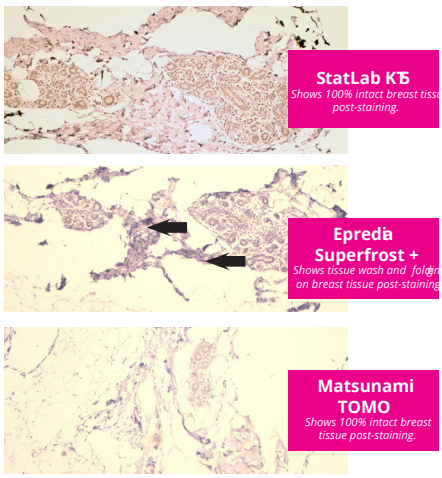

Table 1 Scoring Grid

	12		34		5
Spectrophotometer	< 0.01	0.011-0.025	0.026-0.035	0.036-0.045	> .045
Coverage	<10%	25%	50%	75%	100%
GMS Background Staining	100% clear	>75% clear	>50% clear	>25% clear	>10% clear
Adhesion Post Staining	< 10% intact	25% intact	50% intact	75% intact	100% intact

Schematic Diagram of Drop Contact Angles on Hydrophobic and Hydrophilic Slide Surfaces

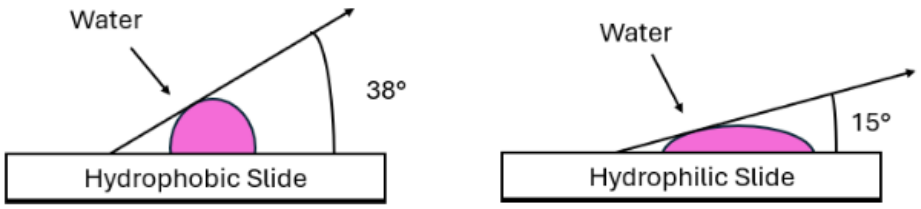

Table 2 Testing Summary

	Waterbath Behavior	Contact Angle	H & E Adhesion	H & E Coverage	H&E Spectrophotometer	GMS Background Staining	IHC Adhesion
Matsunami TOMO	Hydrophilic	35.9	4.85	4.98	5	5	4.82
Dako Flex	Hydrophobic	48.2	NA[1]	NA[1]	4	5	4.67
StatLab KT5+	Hybrid	29.9	4.88	4.95	1	2	4.69
Marienfeld HistoBond	Hydrophobic	49.5	4.86	5.00	2	1	3.87
StatLab MCOMM	Hybrid	38.3	4.98	5.00	2	2	4.38
StatLab KT3+	Hydrophobic	58.6	4.93	4.71	2	3	4.39
StatLab M2000	Hydrophilic	24.2	4.98	4.95	3	3	4.40
StatLab M1000	Hydrophobic	39.9	4.90	5.00	1	1	4.27
StatLab InkPro+	Hydrophobic1	7.5	4.81	5.00	2	1	4.25
Epredia Superfrost+	HydrophobicN	A[2]	4.83	5.00	2	3	4.07
StatLab Colorview+	Hydrophobic	46.8	4.86	4.90	2	3	4.15

IHC Tissues Tested Per Brand (N: ~100)
H & E Tissues Tested Per Brand(N: 21)
See table 1 for scoring definition

Background staining measured 1-5, 1 is the lowest.

Adhesion measured 1-5, 5 is the highest.

1. Dako Flex slides were unable to be procured in a timely manner for the H & E staining portion of study and are excluded from the H & E staining results.
2. Superfrost+ were not analyzed for contact angle measurement.

Table 3 IHC Statistical Analysis

	OverallS	tandard Deviation	Coefficient of Variation
Matsunami TOMO	4.82	0.63	0.13
Dako Flex	4.67	0.63	0.14
StatLab KT5+	4.69	0.87	0.19
Marienfeld HistoBond	3.87	1.56	0.40
StatLab MCOMM	4.38	1.06	0.24
StatLab KT3+	4.39	1.12	0.26
StatLab M2000	4.41	.130	.26
StatLab M1000	4.27	1.11	0.26
StatLab InkPro+	4.25	1.35	0.32
Epredia Superfrost+	4.07	1.20	0.30
StatLab Colorview+	4.15	1.35	0.32

IHC Tissues Tested Per Brand (N: ~100)

Table 4 IHC Failure Rates for Tissue Types

	Overall Failure	EasyM	edium H	ard
Matsunami TOMO	2%	0%	5%	0%
Dako Flex	9%	0%	0%	22%
StatLab KT5+	8%	0%	5%	19%
Marienfeld HistoBond	30%	6%	18%	81%
StatLab MCOMM	22%	3%	7%	69%
StatLab KT3+	16%	0%	5%	47%
StatLab M2000	21%	0%	5%	68%
StatLab M1000	26%	0%	23%	65%
StatLab InkPro+	20%	0%	9%	50%
Epredia Superfrost+	29%	0%	9%	61%
StatLab Colorview+	25%	0%	10%	69%

IHC Tissues Tested Per Brand (N: ~100)
Failure Rate: a slide which lost 50% or more tissue during staining
Easy Difficulty Tissues Tested Per Brand (N: ~30)
Medium Difficulty Tissues Tested Per Brand (N: ~40)
Hard Difficulty Tissues Tested Per Brand (N: ~30)
Any slide which scored at a 1,2, or 3 out of 5 for tissue loss was considered a failure

CONCLUSION

After wide-ranging testing of adhesion slide characteristics, this study shows that not all adhesion slides are created equal. While water bath behavior showed to not be relevant, there is considerable variation in background staining and tissue adhesion between slides. The results of this study suggest to labs that it is important to determine what the needs are for your laboratory based on the types of staining done and tissue types used, and test adhesion slides to find the right slide for your lab. The Matsunami TOMO and Dako Flex slides exhibited the strongest adhesion, but also had the least desirable background staining scores. The StatLab KT5+ scored similarly to TOMO and Dako Flex for adhesion, however background staining scores indicated minimal excess stain on the slide.

Disclaimer: The findings and conclusions presented are based on internal research conducted by our team. The results should be considered preliminary and are not intended to replace external studies or peer-reviewed research. Further validation through additional external studies and peer-reviewed publications should be considered.

REFERENCES

1. https://elearn.nsh.org/products/slide-surface-chemistry-understanding-an-essential-link-to-obtaining-quality-ihc-staining-results
2. https://www.biosb.com/wp-content/uploads/Final-Hydrophilic-Plus-Slides-for-Molecular-Pathology.pdf - source for blurb on contact angle measurement
3. https://www.leicabiosystems.com/us/knowledge-pathway/an-introduction-to-specimen-processing/