FFPE HSV-1 and HSV-2 simulated tissue sections mounted on slides for use as prospective quality controls in IHC and qPCR workflows

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INTRODUCTION

HSV-1(HHV-1) and HSV-2(HHV-2), are double-stranded DNA viruses from the Herpesviridae family. Approximately 90% of people worldwide are infected with HSV-1, HSV-2, or both. In immunocompromised individuals, HSV infections can lead to severe conditions like esophagitis, hepatitis, pneumonia, encephalitis, cancer, and neurodegenerative disorders. HSV infections are diagnosed via culture, immunoassays, immunohistochemistry (IHC), and molecular assays (PCR, sequencing). Retrospective molecular screening using formalin-fixed, paraffin-embedded (FFPE) samples is gaining popularity, but variability in manual processing and a lack of reproducible controls hinder reliable conclusions.

MATERIALS & METHODS

Microbix created reliable simulated specimens to resemble HSV-1 and HSV-2 infected tissues. Cell cultures (BSC-1) were infected with HSV-1 (MacIntyre) and HSV-2 (MS), inactivated via gamma irradiation, mixed with a proprietary ratio of human fibroblast cells (MRC-5), formalin-fixed, and embedded in paraffin blocks that were cut in 5 µm sections. Slides were evaluated for homogeneity (H&E section staining, cell count comparison) and tested using laboratory-developed IHC workflows (HSV 1/2 Antibody PA1-7488, Biotin) and reflex real-time PCR testing (off-label use of Savanna HSV 1+2/VZV Assay, QuidelOrtho Corporation).

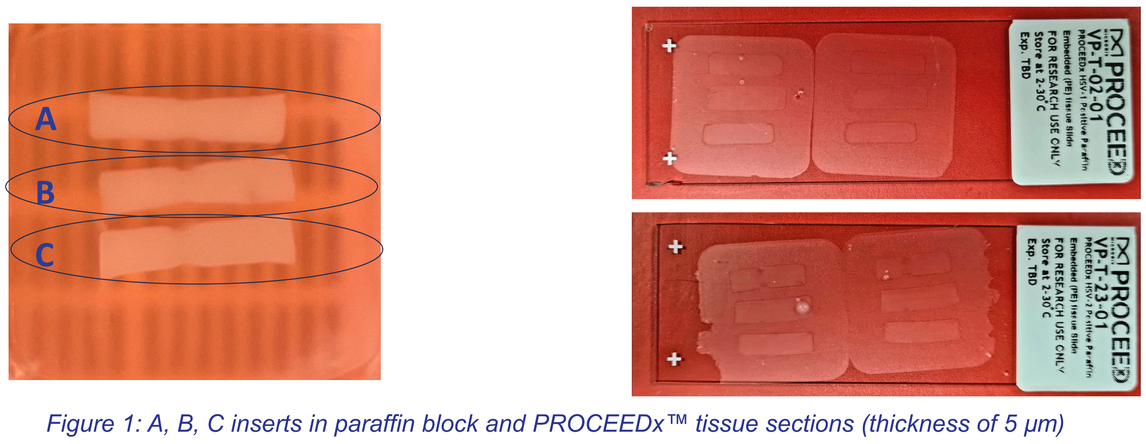

Figure 1: A, B, C inserts in paraffin block and PROCEEDx™ tissue sections (thickness of 5 µm)

Objective 1 Methods – Design Homogeneous HSV-Positive Simulated Specimens

- A proprietary block preparation method was used to produce three simulated tissue inserts per section. The slides sections were labelled as “A”, “B”, and “C” for subsequent homogeneity analysis **(Figure 1).**
- Slides were H&E stained.
- Cell counting software (CardioGenics Inc.) was used for homogeneity analysis. Shapiro-Wilks and ANOVA statistical analyses were conducted to test for normality and calculate significant differences in cell count across all slides, respectively (alpha set at 0.05).

Objective 2 Methods – Design Suitable QC Material for IHC and PCR workflows

- HSV-1 positive and HSV-2 positive sections (simulated patient specimens) were prepared for immunohistochemistry (IHC) using a standard antigen retrieval protocol.
- IHC was conducted on HSV-1 positive and HSV-2 positive sections (HSV 1/2 Antibody PA1-7488, Biotin/ SA-HPR detection).
- Reflex nucleic acid amplification testing was conducted thereafter. Sections were submersed in 1 mL of proprietary DxTM™ (Microbix Biosystems Inc.) and heated in a water bath at 80°C for 30 minutes. 0.3 mL of the preparation was tested on Savanna HSV 1+2/VZV Assay, QuidelOrtho Corporation.

RESULTS

1. Homogeneity Assessment

A. Cell Counting – Descriptive Statistics

Slide ID (A,B,C sections)	Average cell count (Avg. A,B,C)	SD	CV (%)	Total cell count (Sum A,B,C)	Total Average	Total SD	Total CV%
1st slide HSV-1	598.67	79.59	13.29%	1796	1642.17	111.23	6.77%
10th slide HSV-1	577.33	45.98	7.96%	1732			
20th slide HSV-1	534.67	43.00	8.04%	1604			
30th slide HSV-1	550.33	57.50	10.45%	1651			
40th slide HSV-1	529.33	7.37	1.39%	1588			
50th slide HSV-1	494.00	15.72	3.18%	1482			
1st slide HSV-2	568.67	124.11	21.83%	1706	1545.67	94.62	6.12%
10th slide HSV-2	533.33	110.19	20.66%	1600			
20th slide HSV-2	504.00	47.84	9.49%	1512			
30th slide HSV-2	479.00	45.83	9.57%	1437			
40th slide HSV-2	508.67	44.86	8.82%	1526			
50th slide HSV-2	497.67	44.00	8.84%	1493			

Table 1: HSV 1/2-Positive paraffin blocks are cut into sections with consistent cell counts

RESULTS (CONTINUED)

B. H&E Staining and Cell Counting – Inferential Statistics

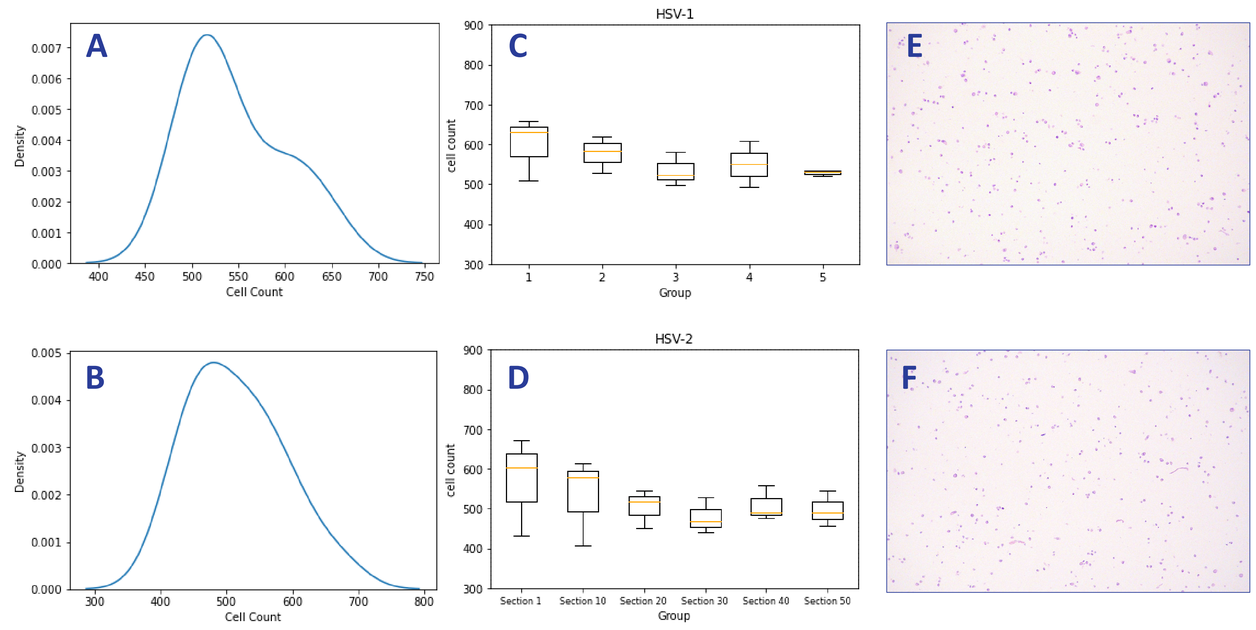

*Figure 2: **(A)** Kernel Density Estimation Plot of HSV-1 positive samples (S=0.914, p=0.101), **(B)** Kernel Density Estimation Plot of HSV-2 positive samples (S=0.967, p=0.742), **(C)** HSV-1 positive slide cell count (f=1.78, p= 0.1916), **(D)** HSV 2-positive slide cell count (f=0.50, p=0.7715), **(E)** HSV-1 cell line 10x, **(F)** HSV-2 cell line 10x*

2. Prospective Histology and PCR Workflows Used to Detect HSV-Positive Samples

A. IHC - HSV 1/2 Antibody PA1-7488, Biotin/ SA-HPR:DAB detection

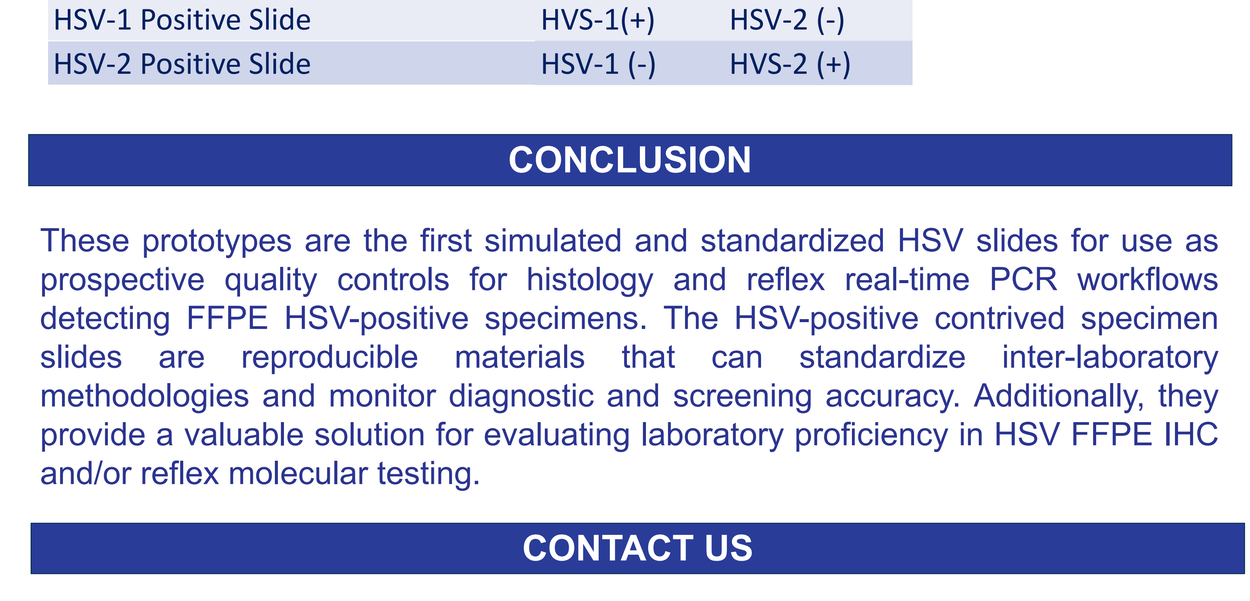

*Figure 3: HSV antigen detection with HSV-1 **(A)** and HSV-2 **(B)** cell line, **(C&D)** Negative control (10x)*

B. Performance on Savanna HSV 1+2/VZV Assay

Sample (n=3)	Outcome	
HSV-1 Positive Slide	HVS-1(+)	HSV-2 (-)
HSV-2 Positive Slide	HSV-1 (-)	HVS-2 (+)

CONCLUSION

These prototypes are the first simulated and standardized HSV slides for use as prospective quality controls for histology and reflex real-time PCR workflows detecting FFPE HSV-positive specimens. The HSV-positive contrived specimen slides are reproducible materials that can standardize inter-laboratory methodologies and monitor diagnostic and screening accuracy. Additionally, they provide a valuable solution for evaluating laboratory proficiency in HSV FFPE IHC and/or reflex molecular testing.

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