36 DIGITAL WHOLE SLIDE IMAGE (WSI) SCORING IS EQUIVALENT TO MICROSCOPE GLASS SLIDE SCORING FOR EVALUATION OF PROGRAMMED DEATH-LIGAND 1 (PD-L1) EXPRESSION ACROSS MULTIPLE TUMOR INDICATIONS

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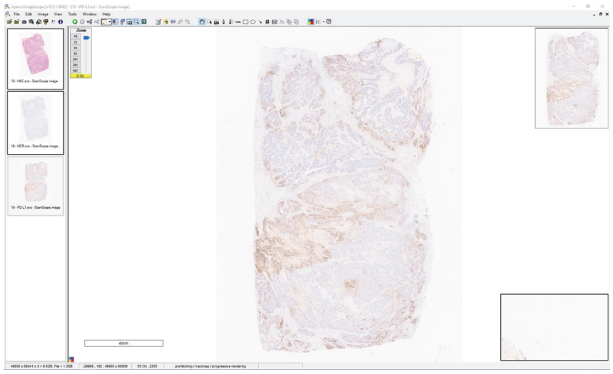
Background The COVID-19 pandemic brought a host of new challenges, including the immediate need for digital solutions addressing the lack of remote options available to pathologists in the field of immunohistochemistry (IHC)-based companion diagnostics for Programmed Death-Ligand 1 (PD-L1) expression evaluation in tumor tissues. Agilent Technologies, Inc. investigated concordance of PD-L1 expression results recorded by trained pathologists between stained glass slides and digital whole slide images (WSIs). Formalin-fixed, paraffin-embedded (FFPE) specimens of eleven tumor indications (table 1) were evaluated in this study. Specimens were stained using the qualitative IHC assay PD-L1 IHC 22C3 pharmDx on Autostainer Link 48 and scored using TPS (Tumor Proportion Score) or CPS (Combined Positive Score) algorithms at six validated cutoffs.[1] The objective was to demonstrate equivalency between digital WSI and microscope glass slide scoring.



Abstract 36 Figure 1 Digital WSI of a triple-negative breast carcinoma (TNBC) specimen stained with PD-L1 IHC 22C3 pharmDx primary antibody and viewed on Aperio ImageScope software with corresponding H&E and NCR WSIs for use as aids in the interpretation of PD-L1 staining.*
*Tissue sample supplied by BioIVT (Hicksville, NY, USA)

Abstract 36 Table 1 Algorithm-cutoff pairs tested

Algorithm	Cutoff	# of Tumor Indications Tested	Tumor Indications Tested/ # of Specimens Tested from Each Indication
CPS	1	8	Urothelial carcinoma (UC): 30 Head and neck squamous cell carcinoma (HNSCC): 32 Cervical cancer: 34 Breast carcinoma (BC) including triple-negative breast carcinoma (TNBC): 37 Renal cell carcinoma (RCC): 30 Biliary tract adeno cancer (BTAC): 32 Small cell lung cancer (SCLC): 30 Colorectal carcinoma (CRC): 38
	10	5	Esophageal cancer: 30 UC: 30 Cervical cancer: 34 BC (including TNBC): 30 BTAC: 32
	20	1	HNSCC: 122
	50	1	HNSCC: 122
TPS	1%	2	Non-small cell lung cancer (NSCLC): 90 NSCLC cytology: 30
	50%	3	HNSCC: 68 NSCLC: 40 NSCLC cytology: 30

Abstract 36 Table 2 Minimum computer monitor requirements for viewing WSIs on Aperio ImageScope

Component	Aperio Image Hub	eSlide Manager and Aperio Image Analysis Workstation
Display type	LCD (flat panel)	LCD (flat panel)
Screen Resolution	1680(h) x 1050(v) pixels	1680(h) x 1050(v) pixels
Screen size	24-inch	24-inch
Color depth	24-bit	24-bit
Brightness	250cd/m² or greater	250cd/m² or greater
Contrast ratio	500:1	500:1

Abstract 36 Table 3 Glass slide vs. digital WSI NPA/PPA/OA results summary for the six algorithm-cutoff pairs tested

Agreement Summary						
Algorithm	Cutoff	Total Comparisons	Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
					Lower-bound: 2.5%	Upper-bound: 97.5%
CPS	1	358	NPA	96.6%	94.521	98.603
		426	PPA	97.9%	96.487	99.061
		784	OA	97.3%	95.802	98.599
CPS	10	223	NPA	93.3%	89.091	96.847
		243	PPA	95.1%	91.968	97.561
		466	OA	94.2%	91.845	96.360
CPS	20	164	NPA	91.5%	86.310	95.808
		197	PPA	88.3%	83.505	92.746
		361	OA	89.8%	85.912	93.333
CPS	50	179	NPA	91.6%	87.059	95.652
		180	PPA	90%	84.153	95.055
		359	OA	90.8%	86.944	94.167
TPS	1%	171	NPA	90.6%	85.965	94.767
		188	PPA	97.3%	94.149	99.479
		359	OA	94.2%	91.365	96.657
TPS	50%	230	NPA	91.7%	87.611	95.575
		180	PPA	93.9%	89.617	97.340
		410	OA	92.7%	89.731	95.400

Abstract 36 Table 4 Number of PD-L1 expression level discordances in HNSCC CPS ≥20 study for predefined negative, near-cutoff (NCO) negative, NCO positive, and positive categories based on specimen screening data assigned by one or more Agilent pathologists prior to the study

PD-L1 Expression Level Discordances with Respect to Screening Data at CPS ≥20 Cutoff		
Specimen Category with CPS ≥20 Cutoff	# of Discordances Generated by Glass Slide Scoring	# of Discordances Generated by Digital WSI Scoring
Negative	10	8
Negative NCO	15	10
Positive NCO	3	6
Positive	6	5

Abstract 36 Table 5 Number of PD-L1 expression level discordances in HNSCC CPS ≥50 study for predefined negative, NCO negative, NCO positive and positive categories based on specimen screening data assigned by one or more Agilent pathologists prior to the study

PD-L1 Expression Level Discordances with Respect to Screening Data at CPS ≥50 Cutoff		
Specimen Category with CPS ≥50 Cutoff	# of Discordances Generated by Glass Slide Scoring	# of Discordances Generated by Digital WSI Scoring
Negative	2	6
Negative NCO	10	9
Positive NCO	10	13
Positive	2	4

Methods Three Agilent-certified pathologists evaluated specimen PD-L1 expression level (positive/negative) using CPS and/or TPS at relevant cutoff(s) for each indication (table 1) using two scoring modalities for the same specimen sets: 1) light microscope, and, 2) digital monitor (WSI) with a minimum 14-day washout period between glass slide and WSI reads. WSIs were generated using Leica's Aperio AT2 scanner and evaluated using Aperio ImageScope software (figure 1) on appropriate monitors (table 2). Concordance between specimen glass slide (reference condition) and WSI PD-L1 expression results was assessed per cutoff on pooled data from all applicable indications using negative percent agreement (NPA),

positive percent agreement (PPA) and overall agreement (OA) with 95% two-sided percentile bootstrap confidence intervals (CI); the acceptance criteria for equivalency at each cutoff were set at CI lower-bounds (CILBs) ≥85%. Discordant comparisons with respect to specimen screening data generated prior to inclusion in the study were also analyzed where applicable.

Results NPA/PPA/OA CILBs for the CPS ≥1, CPS ≥10, TPS ≥1%, and TPS ≥50% cutoffs were ≥85% (table 3). NPA and OA CILBs at CPS ≥20 and CPS ≥50 were ≥85%; PPA CILBs were 83.2% and 84.2%, respectively. Discordant comparisons analysis for CPS ≥20 and CPS ≥50 suggested that WSI is not more prone to discordances in PD-L1 expression level than glass slide scoring when compared to specimen screening data (tables 4 and 5).

Conclusions Glass slide and WSI scoring are equivalent across multiple validated cutoffs and tumor indications tested for PD-L1 expression using PD-L1 IHC 22C3 pharmDx with CPS and/or TPS algorithms and are, thus, considered interchangeable scoring modalities.

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Trial Registration N/A

REFERENCE

1. P02893/13 Instructions for Use (IFU) for PD-L1 IHC 22C3 pharmDx Human Cancer (SK00621-4) Package Insert

Ethics Approval N/A
Consent N/A

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