

Supplementary Materials: Improving the Diagnostic Accuracy of the PD-L1 Test with Image Analysis and Multiplex Hybridization

Table S1. Antibody and Retrieval Information.

DAB Immunohistochemistry – Ventana Benchmark XT									
Biomark er	Clone	Source	Antigen Retrieval	Dilution	Incubatio n	Detection chemistry	Control tissue		
PD-L1	SP263	Ventana	CC1 for 64 minutes	Neat	20 minutes	OptiView DAB	Tonsil		
Opal Multiplex Immunofluorescence – Leica Bond RX									
Step	Biomarker	Clone	Sourc e	Antigen Retrieval	Diluti on	Incubati on	Detection chemistry	Incubati on	Control tissue
1	CD8	C8/144 B	Dako	ER2 20 minutes @ 95°C	1:400	30 minutes	Opal 690 @ 1:150	30 minutes	Tonsil
2	PD-L1	SP263	Venta na	ER2 20 minutes @ 95°C	1:2	30 minutes	Opal 520 @ 1:150	30 minutes	Tonsil
3	CD68	514H1 2	Leica	ER2 20 minutes @ 95°C	1:200	30 minutes	Opal 570 @ 1:150	30 minutes	Tonsil
4	CK	AE1/A E3	Dako	ER2 20 minutes @ 95°C	1:100	30 minutes	Opal 620 @ 1:150	30 minutes	Tonsil
5	DAPI	n/a	Perkin Elmer	ER2 20minut es @ 95°C	1:500	10 minutes	n/a	n/a	Tonsil
ULTIVUE Multiplex Immunofluorescence – Leica Bond RX									
Step	Reagent	Clone	Sourc e	Antigen Retrieval	Dilution	Incubatio n	Detecti on chemist ry	Control tissue	
1	Antibody Diluent	n/a	Ultivu e	n/a	neat	15 minutes	n/a	Tonsil	
2	PD-L1, CD8, CD68 & CK	7310, C8/144B, KP-1, & AE1/AE3	Ultivu e	ER2 20 minutes @ 95°C	1:100	60 minutes	n/a	Tonsil	
3	Pre- Amplificati on Mix	n/a	Ultivu e	n/a	neat	25 minutes	n/a	Tonsil	
4	Amplificati on Solution	n/a	Ultivu e	n/a	1:10	90 minutes	n/a	Tonsil	
5	Nuclear Counterstain	n/a	Ultivu e	n/a	1:100	15 minutes	n/a	Tonsil	
6	Fluorescen t Probes	n/a	Ultivu e	n/a	1:20	25 minutes	Cy5, FITC, Cy3, Cy7.	Tonsil	

All incubations at room temperature unless otherwise stated.

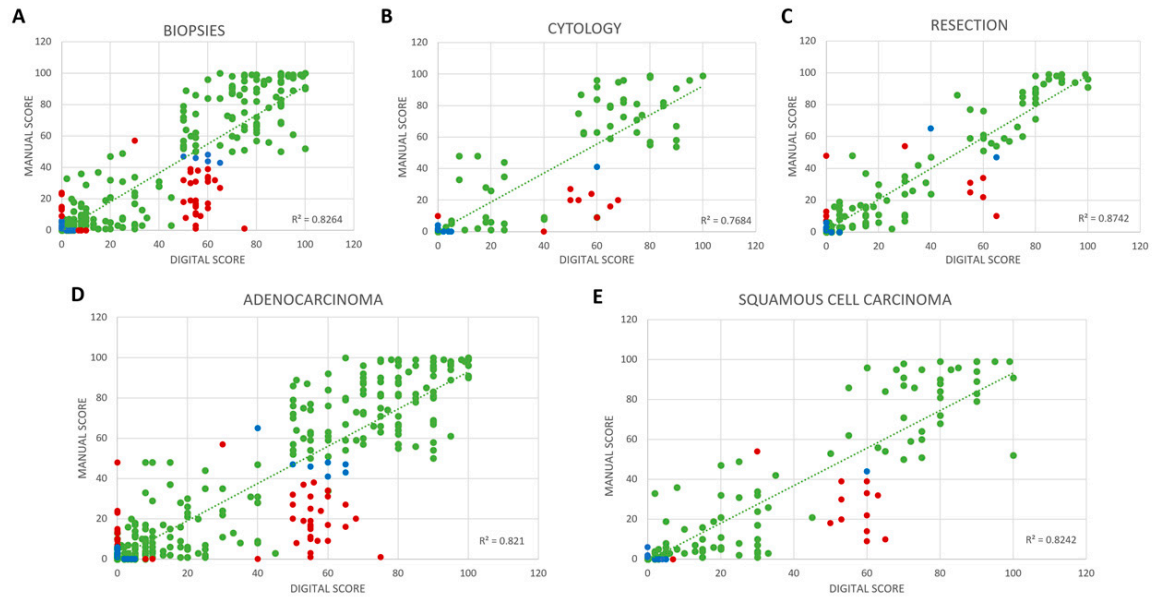


Figure S1. Concordance between Manuel and digital assessment. Concordance between manual and digital assessment by (A) Biopsy (B) Cytology (C) Resections and histology (D) and (E). In all the same types and both adenocarcinoma and squamous cell carcinoma samples there is a high level of concordance.

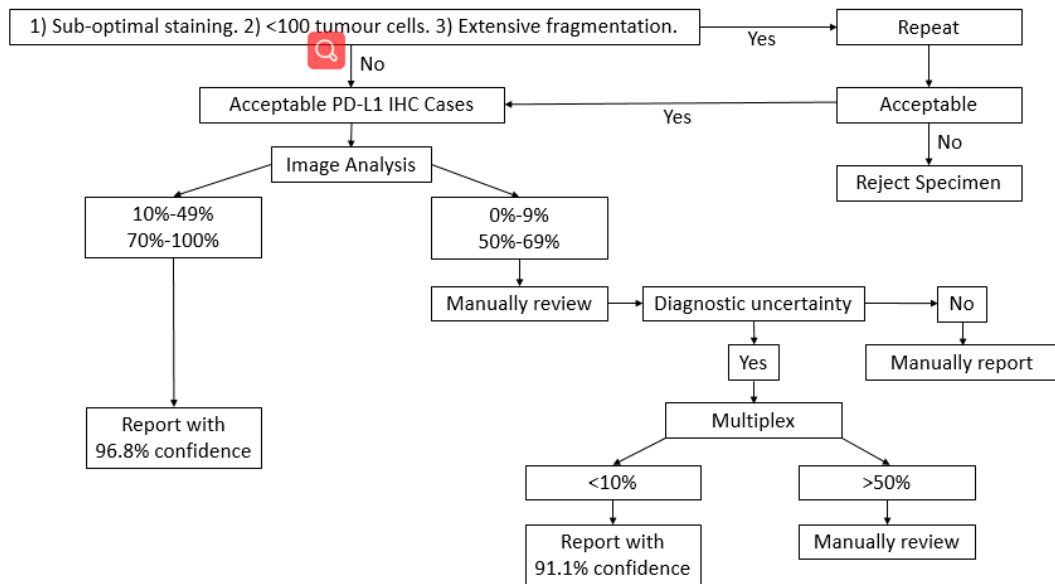


Figure S2. A diagnostic decision tree proposing the most beneficial application of image analysis and multiplex to appropriately triage PD-L1 diagnostic cases.