Lung cancer: PD-L1 testing, NordiQC EQA

Workshop in Diagnostic Immunohistochemistry Oud St. Jan/ Old St. John – Brugge (Bruges), Belgium June 13th – 15nd 2018

Rasmus Røge, MD, NordiQC scheme organizer

NordiQC external QA PD-L1

- Two pilot runs with 10 labs
- All NordiQC participant invited for new ”Companion module”
- First run in Spring 2017
- Three ”official” runs (C1-C3) and 1 supplementary run (C1x)
- Participants from more than 25 countries

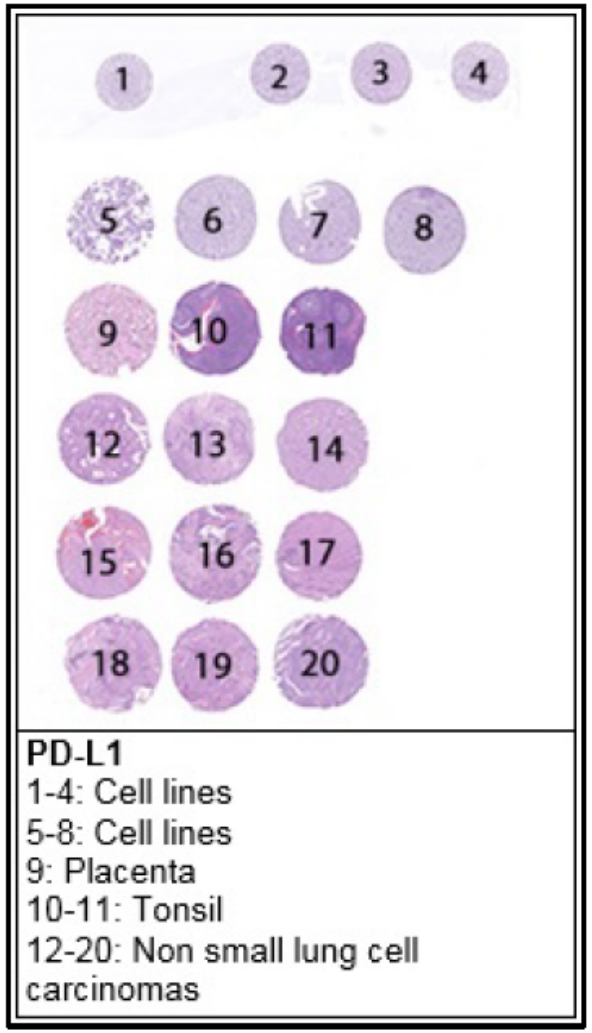
	C1	C2	C3
Participants	68	145	146
Pass rate	50 %	84 %	91 %
Optimal	37 %	59 %	74 %

Development in pass rates

	C1	C2	C3
	68	145	146
Pass rate	50 %	84 %	91 %
Optimal	37 %	59 %	74 %

"However, the assessment of C3 was challenged by less than optimal material circulated (and for a minor fraction of the participant the included NSCLCs displayed varying degrees of PD-L1 expression heterogeneity). Additionally, some slides were missing critical cores. It must be underlined, that no lab was downgraded based on the quality of the circulated slides. However, this decision may have provided overall higher pass rate compared to if the circulated material had been of the required quality."

PD-L1 C2, TMA



NSCLC		
12. NSCLC	No <1%	No
13. NSCLC	No <1%	No
14. NSCLC	No <1%	No
15. NSCLC	Excluded	Excluded
16. NSCLC	Low 1-49%	Yes
17. NSCLC	High ≥50%	Yes
18. NSCLC	High ≥50%	Yes
19. NSCLC	High ≥50%	Yes
20. NSCLC	High ≥50%	Yes

PD-L1, C2 Participant scoring

NordiQC Info ▾ Modules ▾ Assessments Protocols Controls Events ▾ 101

New scoring sheet PD-L1 IHC

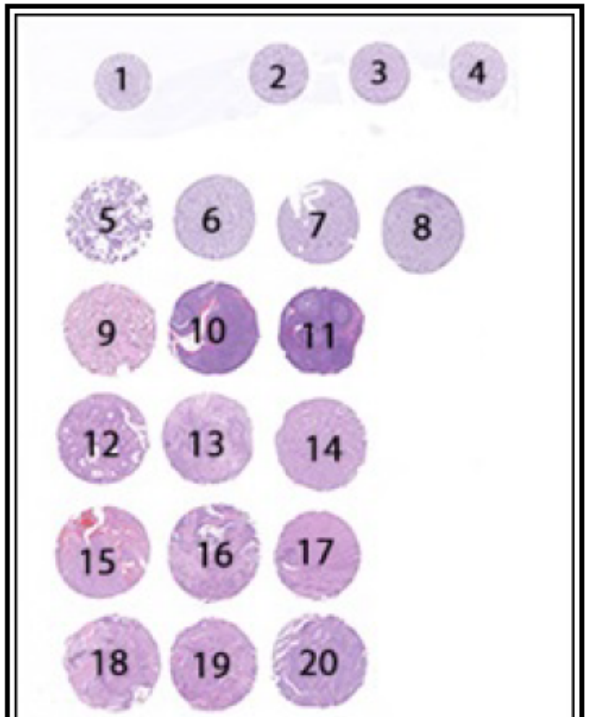
Link to accompany letter

For each NSCLC tumour core percentage of PD-L1 positive tumour cells (viable tumor cells showing partial or complete membrane staining irrespective of staining intensity) should be scored.

Score core 12 (%)	
Score core 13 (%)	
Score core 14 (%)	
Score core 15 (%)	
Score core 16 (%)	
Score core 17 (%)	
Score core 18 (%)	
Score core 19 (%)	
Score core 20 (%)	

Save Back

PD-L1 C2, Assessment



PD-L1
1-4: Cell lines
5-8: Cell lines
9: Placenta
10-11: Tonsil
12-20: Non small lung cell carcinomas

PD-L1 IHC, Technical assessment

Criteria for assessing a staining as Optimal included:
The staining is considered perfect or close to perfect in all of the included tissues.
TPS is concordant to the NordiQC reference data is obtained in all 8 NSCLC cores.

Criteria for assessing a staining as Good included:
The staining is considered acceptable in all of the included tissues. However, the protocol may be optimized to ensure the best staining intensity, counter staining, morphology and signal-to-noise ratio.
TPS is still concordant to the NordiQC reference data in all 8 NSCLC cores.

Criteria for assessing a staining as Borderline included:
The staining is considered insufficient, e.g., because of a generally too weak staining, a false negative staining or a false positive staining reaction of one of the included tissues. The protocol should be optimized.
TPS is **not** found concordant to the NordiQC reference data in all 8 NSCLC cores.

Criteria for assessing a staining as Poor included:
The staining is considered very insufficient e.g., because of a false negative or a false positive staining reaction staining of more of the included tissues.
An optimization of the protocol is urgently needed.
TPS is **not** found concordant to the NordiQC reference data in all 8 NSCLC cores.

Results C2

Table 3. **Assessment marks for IHC assays and antibodies run C2, PD-L1 IHC**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
22C3 pharmDX, **SK006**	23	Dako/Agilent	15	7	0	1	96%	96%
22C3 pharmDX, **SK006**[4]	5	Dako/Agilent	1	2	0	2	60%	-
28-8 pharmDX, **SK005**	6	Dako/Agilent	6	0	0	0	100%	100%
SP263, 790-4905	49	Ventana/Roche	44	2	2	1	94%	98%
SP263, 790-4905[5]	2	Ventana/Roche	0	0	2	0	-	-
Antibodies[3] for laboratory developed PD-L1 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	39	Dako/Agilent	12	18	4	5	76%	-
mAb clone **E1L3N**	9	Cell Signaling	2	6	1	0	89%	-
mAb **CAL10**	2	Biocare	0	1	0	1	-	-
mAb **CAL10**	1	Zytomed	0	0	0	1	-	-
rmAb clone **28-8**	3	Abcam	1	1	1	0	-	-
rmAb clone **ZR3**	1	Zeta Corporation	1	0	0	0	-	-
rmAb clone **ZR3**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **ZR3**	1	Gene Tech	0	1	0	0	-	-
rmAb clone **SP142**	1	Spring Biosystems	0	0	1	0	-	-
rmAb clone **QR1**	1	Quartett	1	0	0	0	-	-
rmAb clone **HDX3**	1	Halioseek	1	0	0	0	-	-
Total	145		85	38	11	11	-	-
Proportion			59%	26%	8%	8%	85%	-

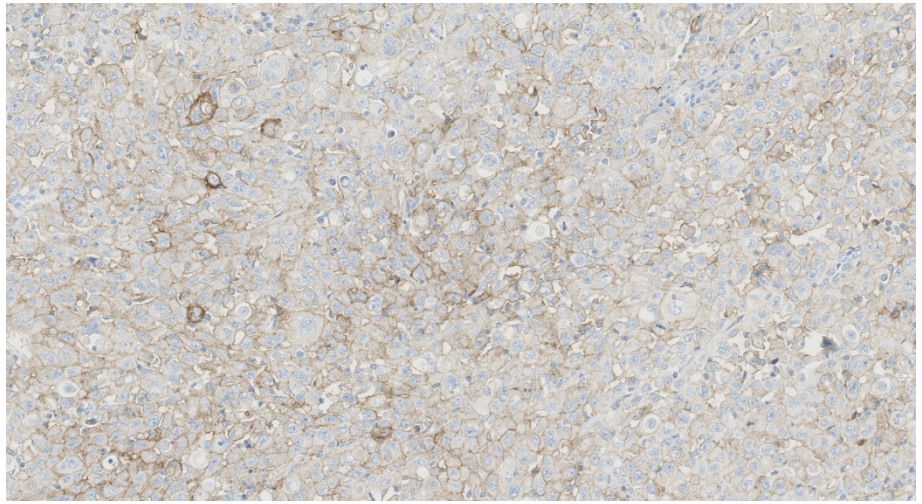
1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.
4) RTU system developed for the Agilent/Dako`s semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Ventana Benchmark and Dako Omnis).
5) RTU system developed for the Ventana/Roche`s automated systems (BenchMark) but used by laboratories on different platforms (Leica Bond and Dako AS48).

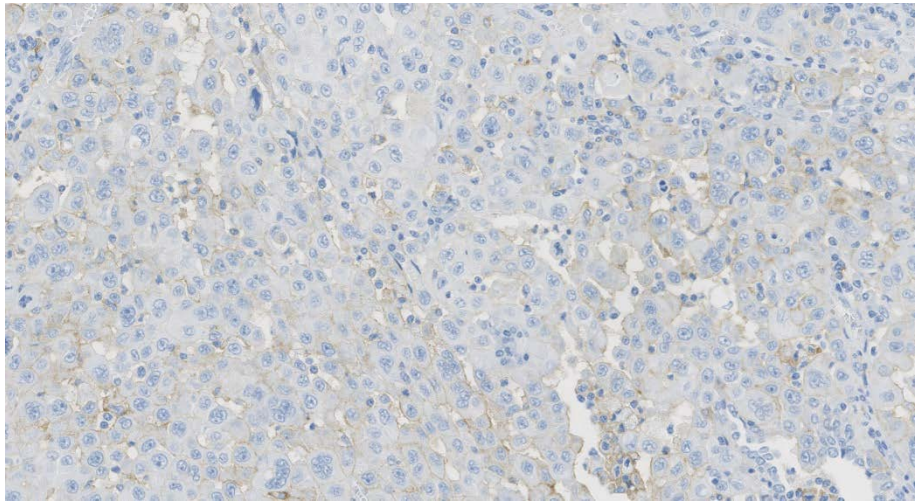
NordiQC

Optimal

Insufficient

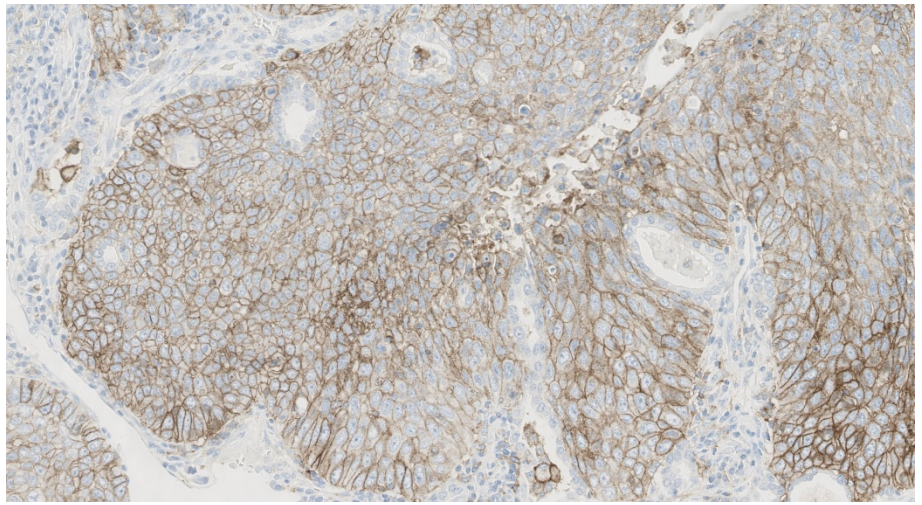
Core 17:
TPS: > 50%

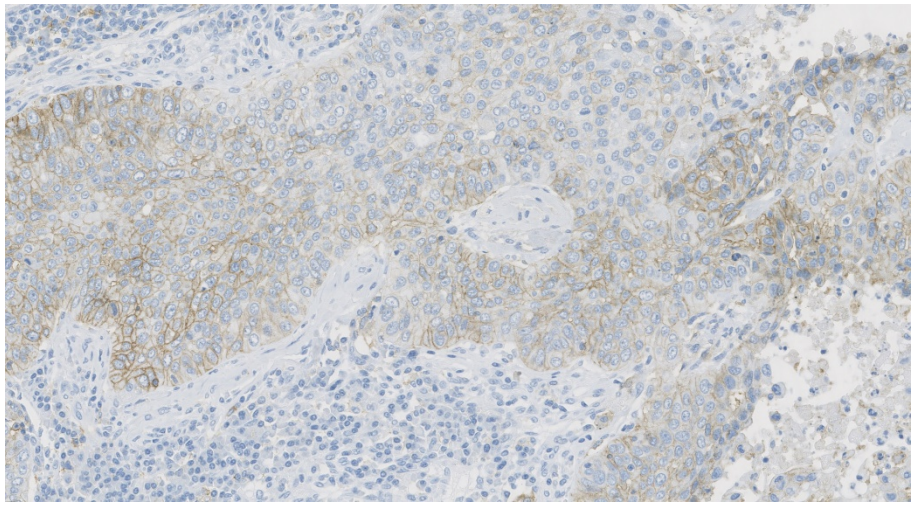




Core 17:
Significant loss of cells

Core 19:
TPS: > 50%





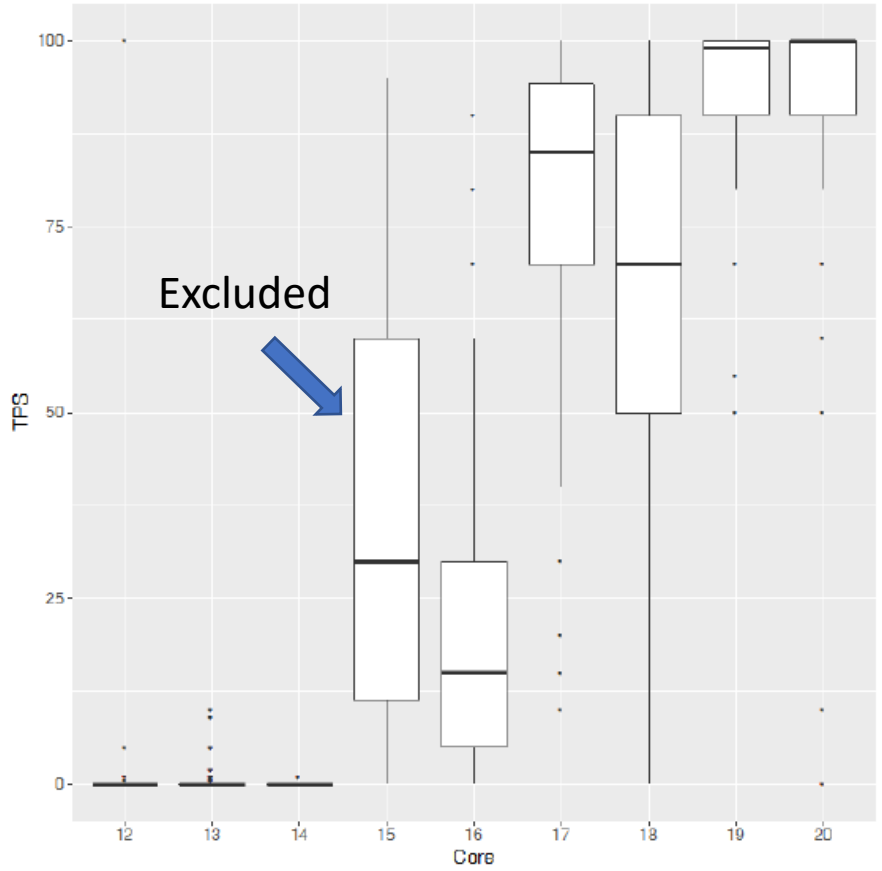
Core 19:
Significant loss of cells

pharmDx IHC PD-L1, SK006, mAb 22C3

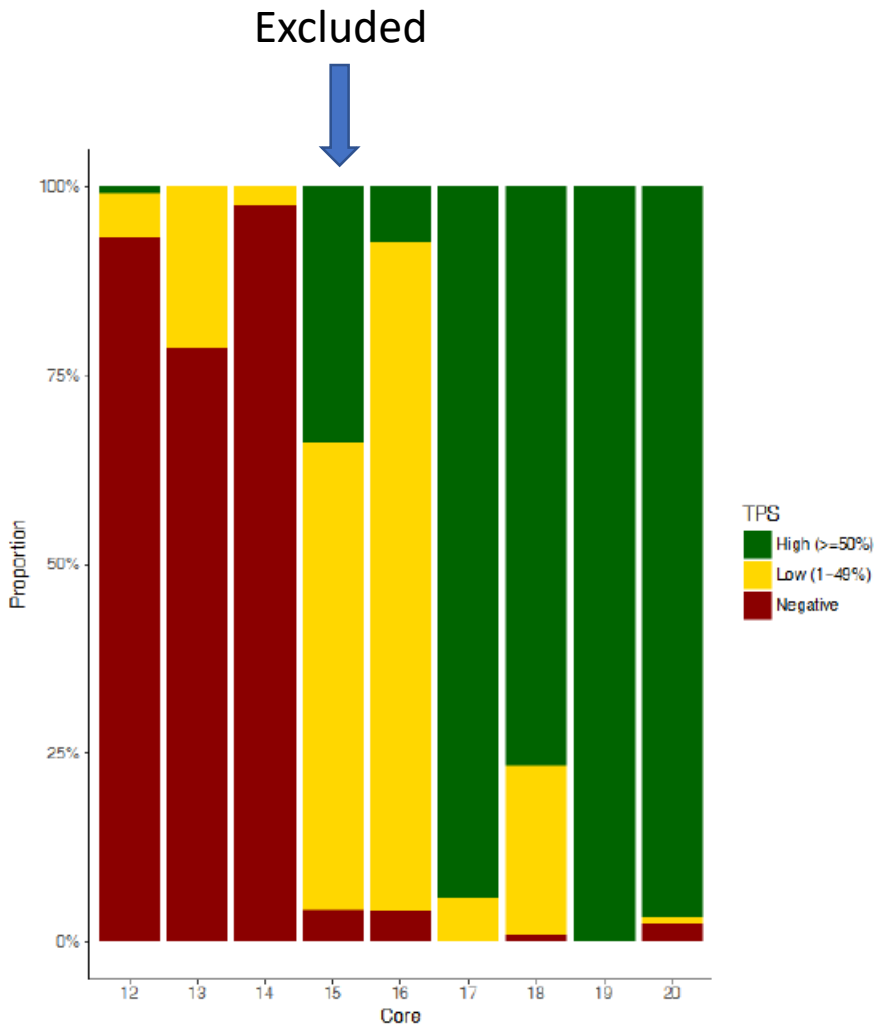
LDT, mAb 22C3, Benchmark

NordiQC

PD-L1, C2 – Interpretation



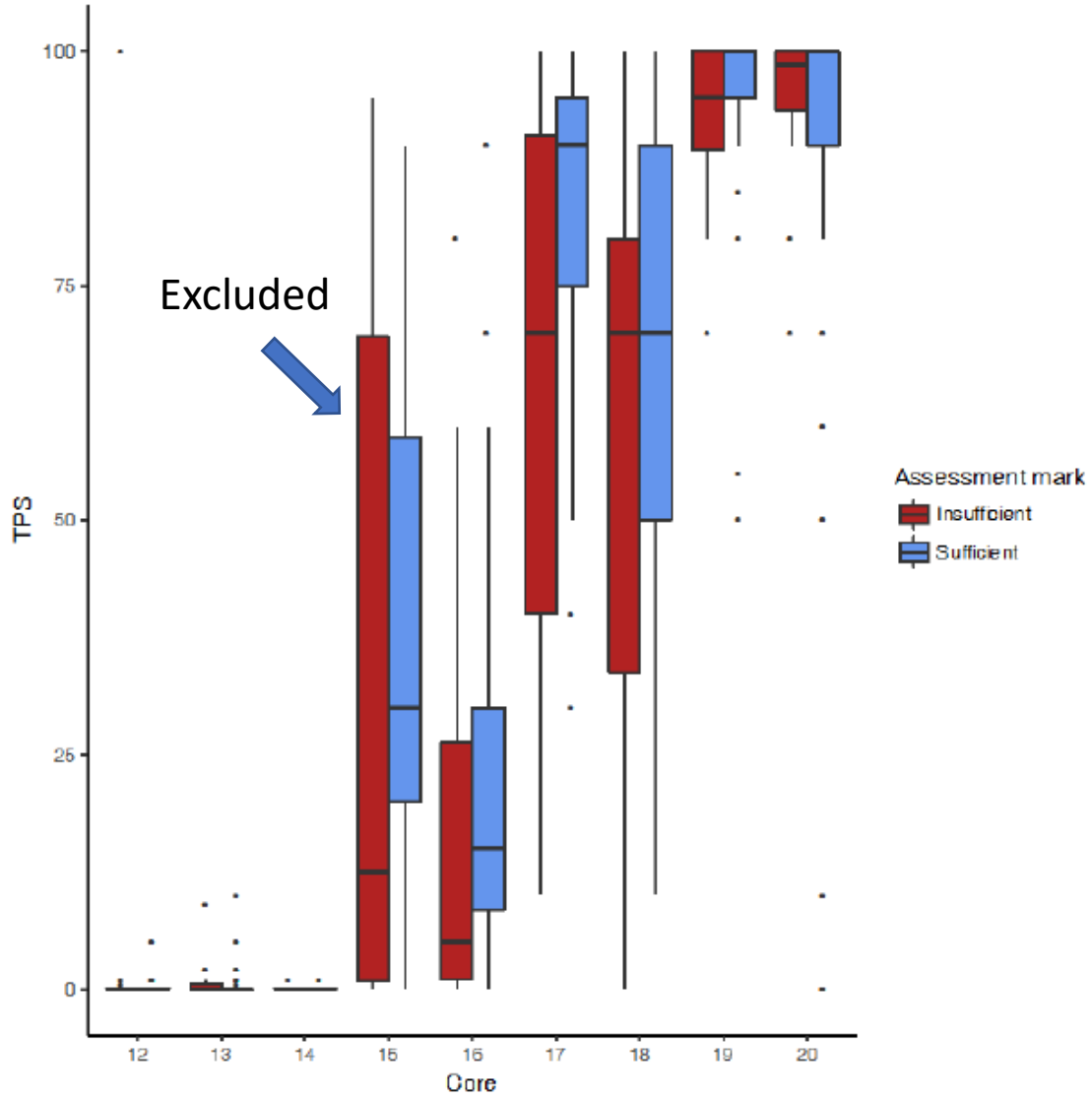
Graph 1. **NordiOC PD-L1 run C2: Participants' TPS scores (interpretation of the percentage of positive tumour cells).**



Graph 2. **NordiQC PD-L1 run C2: participant interpretation of PD-L1 TPS – impact on treatment**

PD-L1, C2 – Interpretation

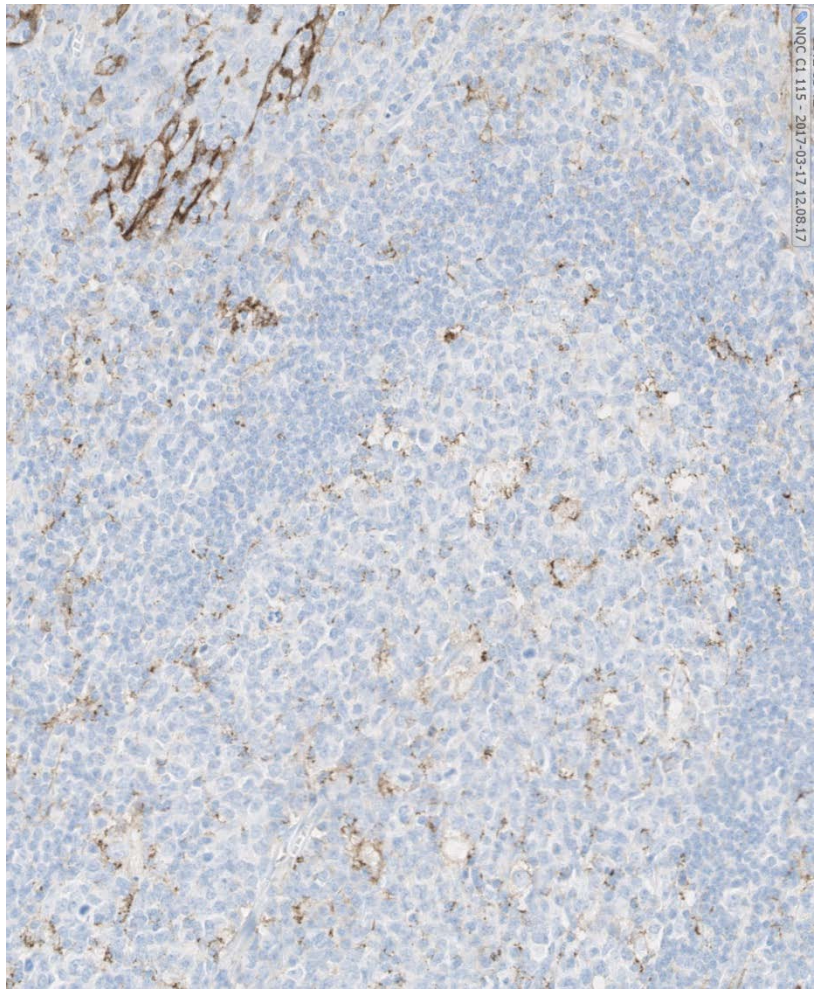
Sufficient vs. insufficient



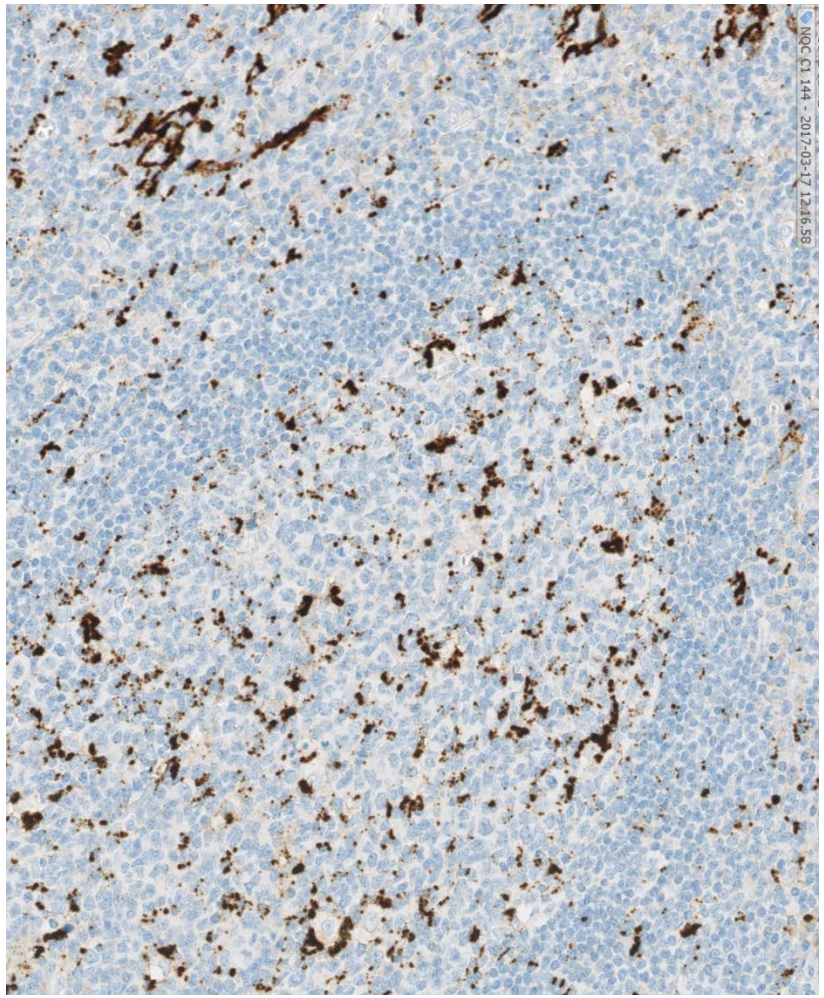
Graph 3. **NordiQC PD-L1 run C2: interpretation concordance for labs with sufficient vs. insufficient results**

NordiQC

PD-L1 staining with TSA (tonsil)



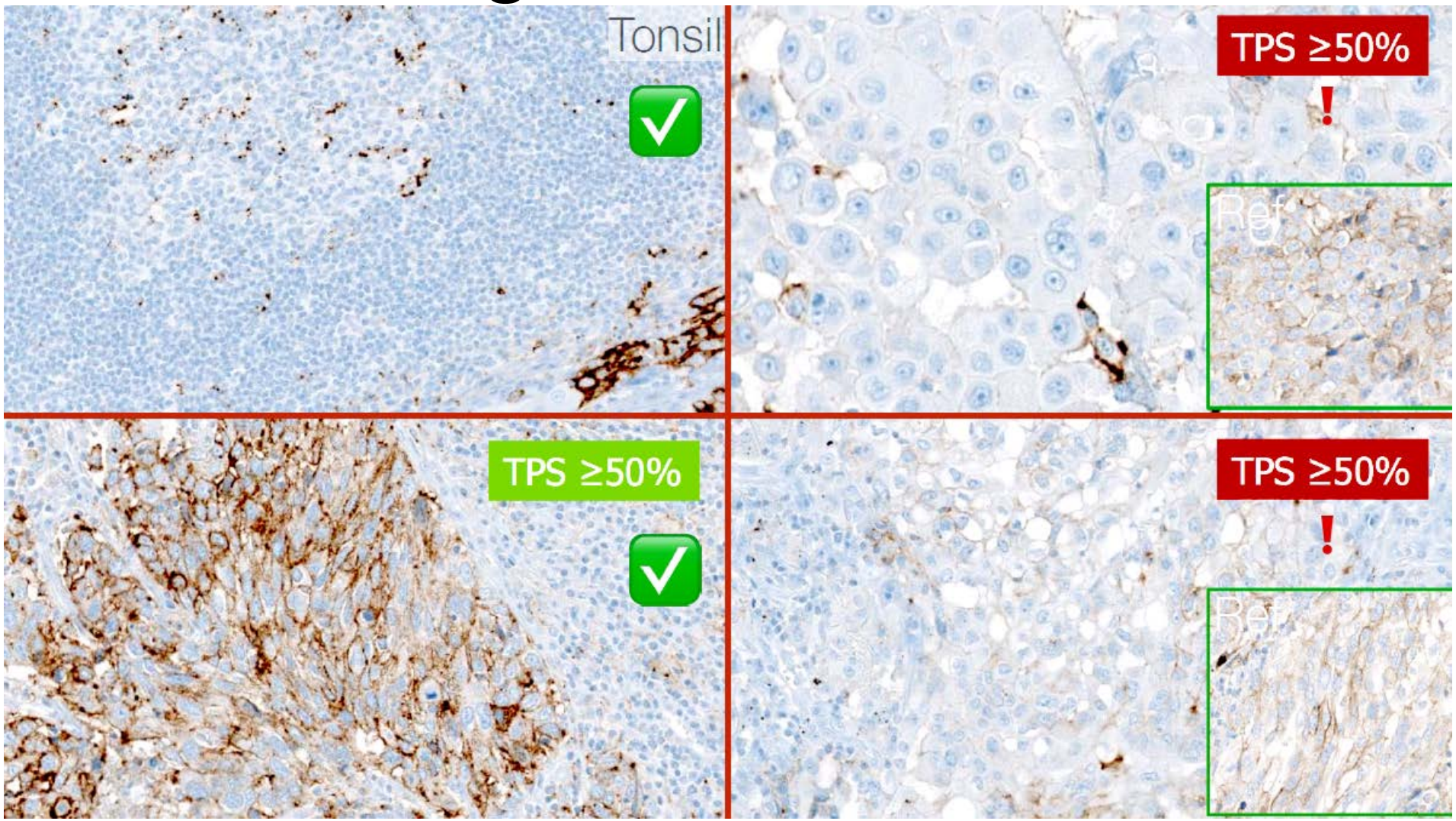
Optimal staining without amplification



Optimal staining with amplification

NordiQC

PD-L1 staining with TSA



LDT protocol:
mAb 22C3 conc
Ventana Bencmark
Optiview
Tyramide amp.

Courtesy of O. Nielsen

NordiQC

Results C3

Table 3. **Assessment marks for IHC assays and antibodies run C3, PD-L1 IHC**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
SP263, **790-4905**	52	Ventana/Roche	41	6	5	0	90%	92%
SP263, **790-4905**[3]	1	Ventana/Roche	1	0	0	0	-	-
22C3 pharmDX, **SK006**	27	Dako/Agilent	22	3	0	2	93%	100%
22C3 pharmDX, **SK006**[4]	8	Dako/Agilent	2	4	1	1	75%	-
28-8 pharmDX, **SK005**	5	Dako/Agilent	4	1	0	0	100%	100%
SP142, **740-4859**[5]	1	Ventana/Roche	0	0	0	1	-	-
Antibodies[6] for laboratory developed PD-L1 assays, conc. antibody		Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	32	Dako/Agilent	27	4	1	0	97%	100%
mAb clone **E1L3N**	6	Cell Signaling	3	3	0	0	100%	100%
mAb **CAL10**	2 3	Biocare Zytomed Systems	1	2	1	1	60%	100%
rmAb clone **28-8**	3	Abcam	3	0	0	0	-	-
rmAb clone **ZR3**	1 1	Cell Marque Zeta Corporation	2	0	0	0	-	-
rmAb clone **QR1**	2	Biocyc	1	1	0	0	-	-
rmAb **BSR90**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **SP142**[5]	1	Spring Biosystems	0	1	0	0	-	-
Total	146		108	25	8	5	-	-
Proportion			74%	17%	6%	3%	91%	-

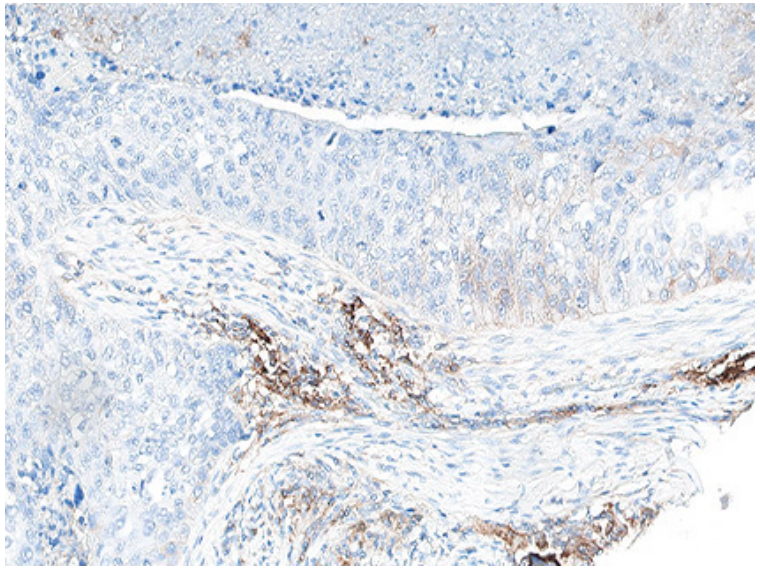
1) Proportion of sufficient stains (optimal or good). 2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Ventana/Roche automated systems (BenchMark) but used by laboratories on a different platform (Leica Bond).
4) RTU system developed for the Agilent/Dako semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Ventana BenchMark and Dako Omnis).

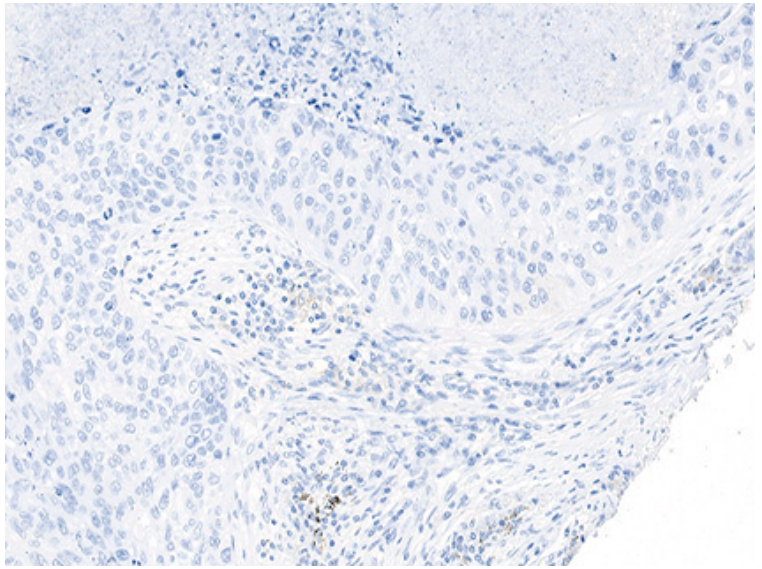
NordiQC

Optimal

Insufficient

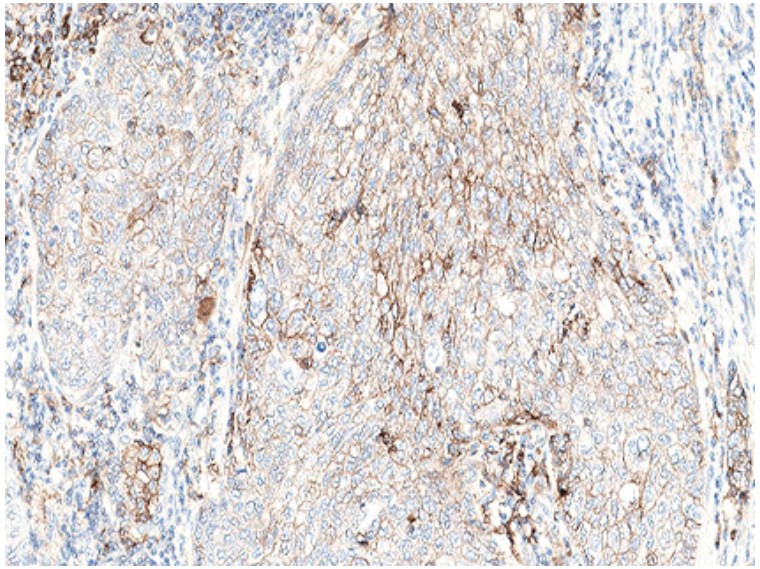
Core 9:
TPS: 1-49%

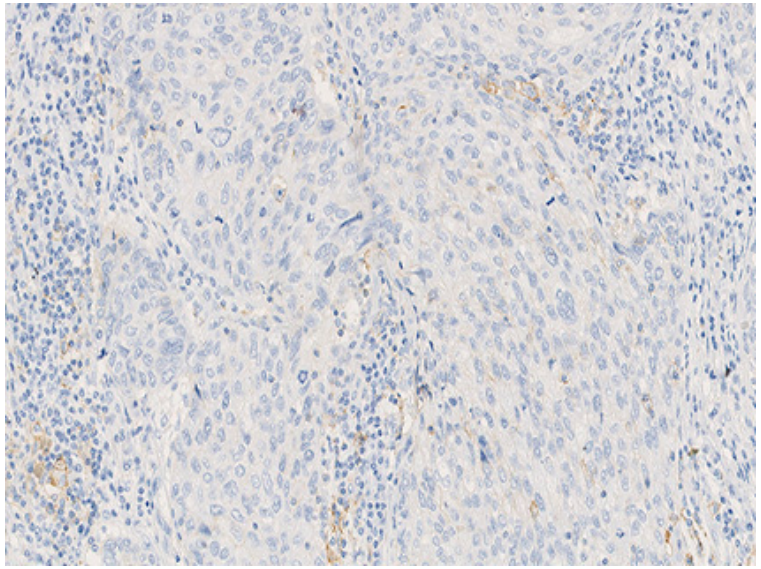




Core 9:
Negative

Core 10:
TPS: > 50%





Core 10:
Significant loss of cells

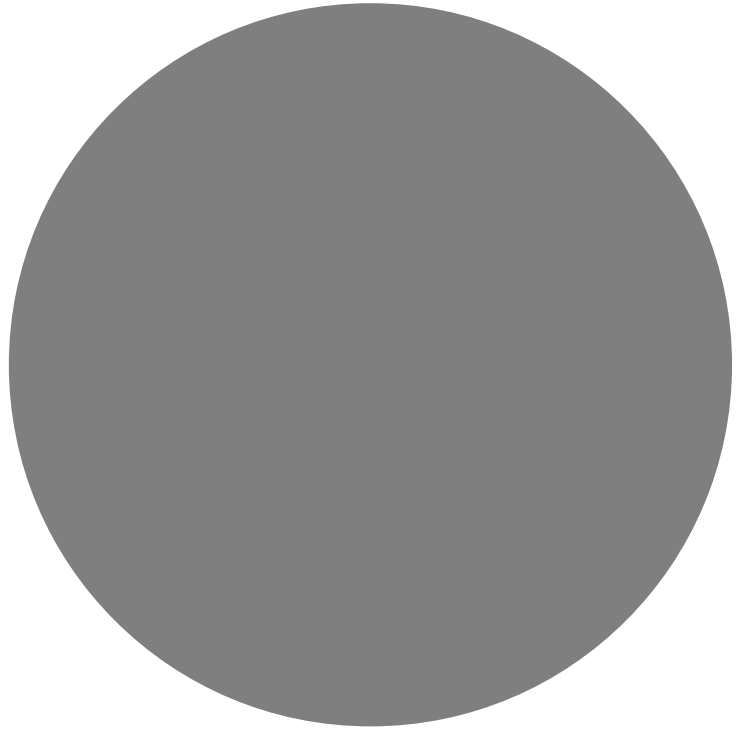
pharmDx IHC PD-L1, SK006, mAb 22C3,
Recommended protocol

pharmDx IHC PD-L1, SK006, mAb 22C3,
Short HIER, Envion

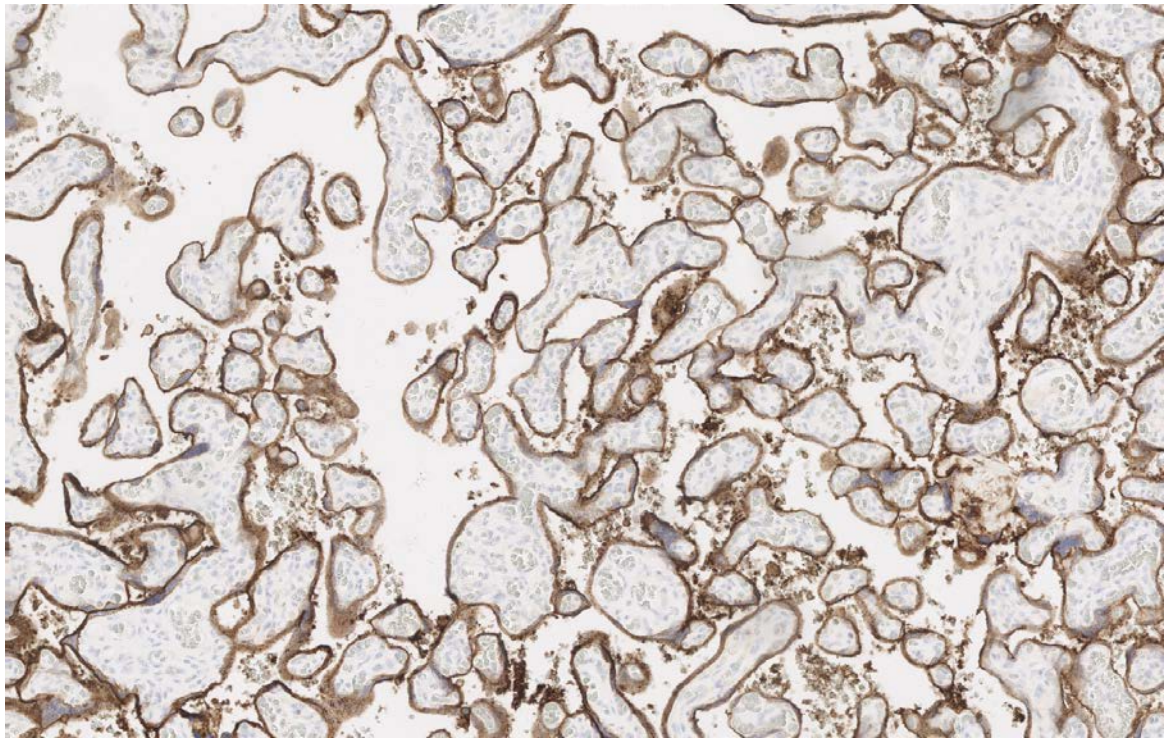
NordiQC

Controls

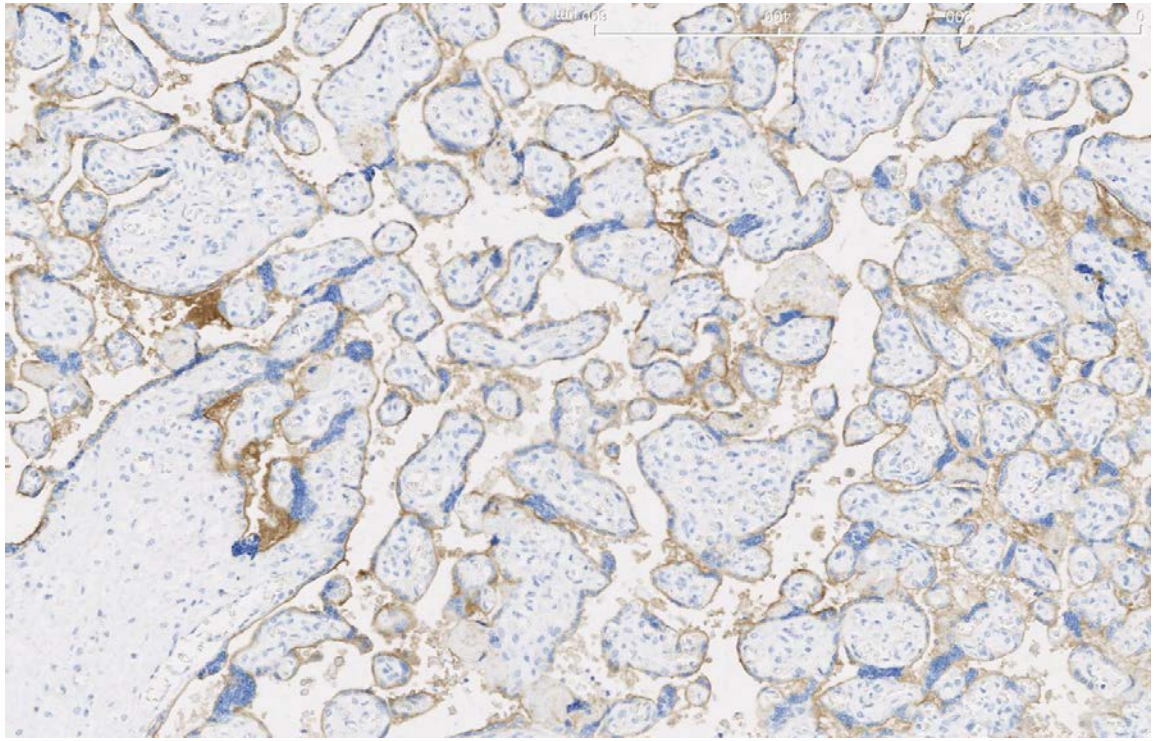
PD-L1, C1-C3



NordiQC



Optimal

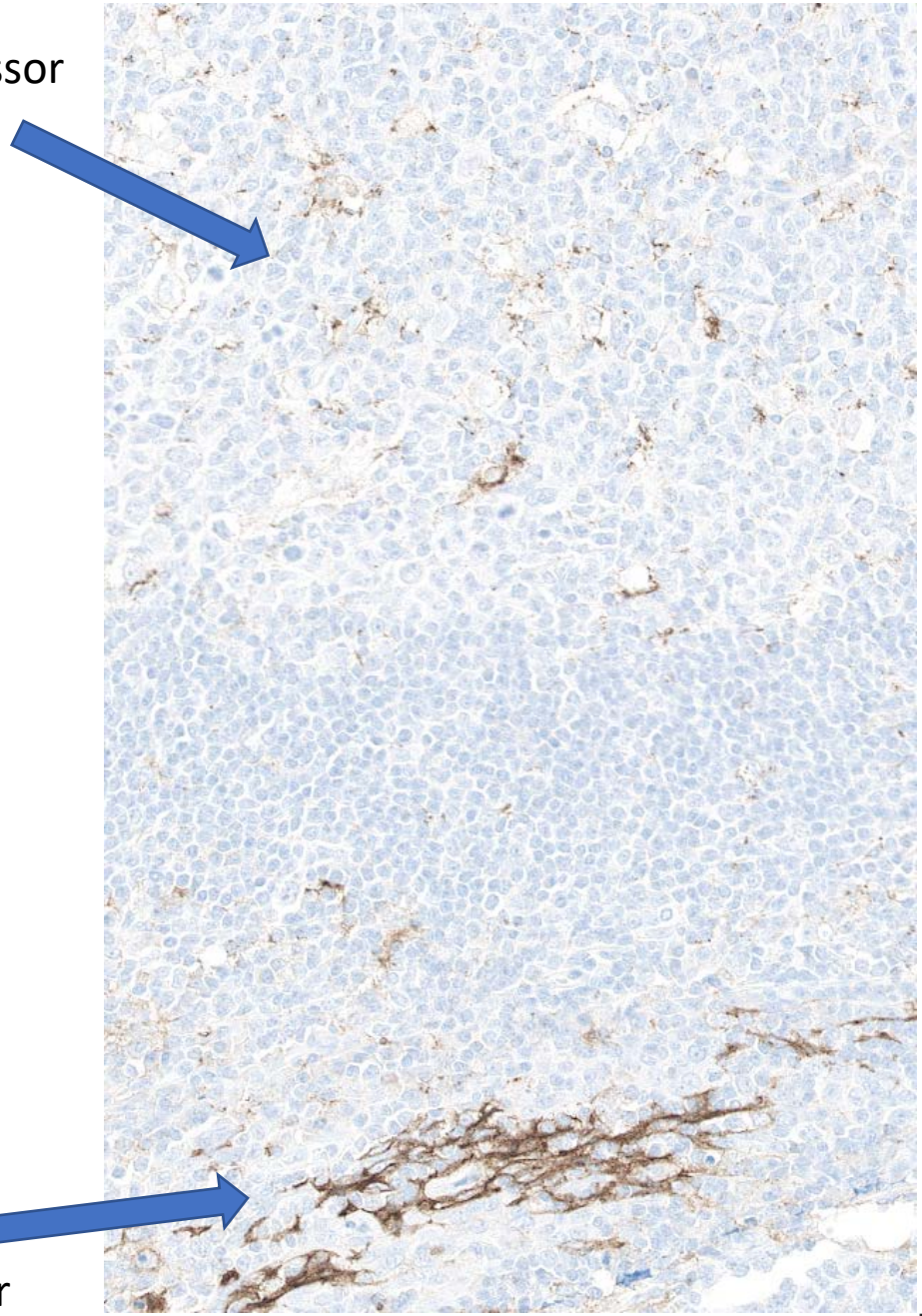


Poor

Placenta

Tonsil

- No staining reaction in the vast majority of lymphocytes including mantle zone and germinal centre B-cells
- A weak to moderate, typically punctuated membranous staining reaction of the majority of germinal centre macrophages
- A moderate to strong staining reaction of the majority of epithelial crypt cells.
- No staining reaction in superficial epithelial cells

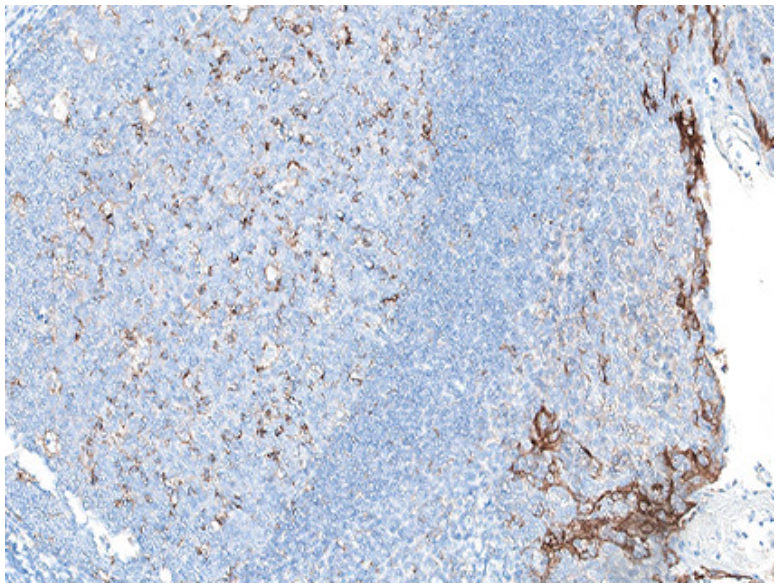


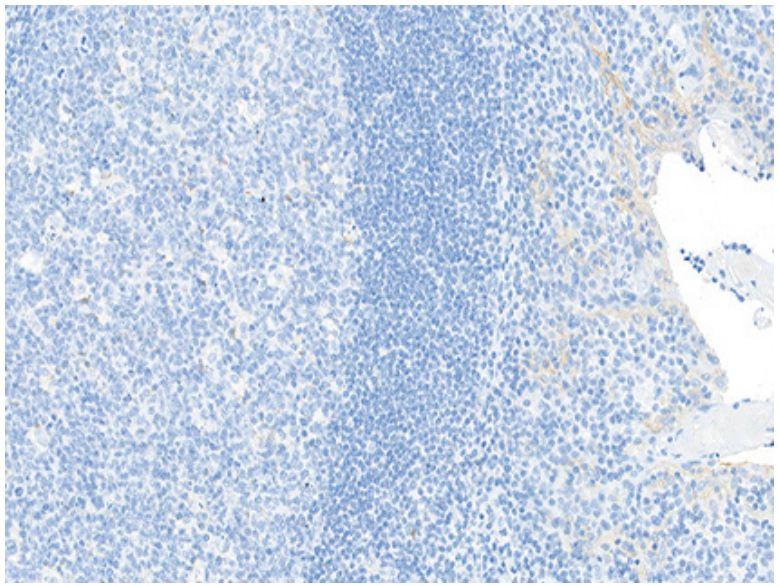
NordiQC

Optimal

Poor

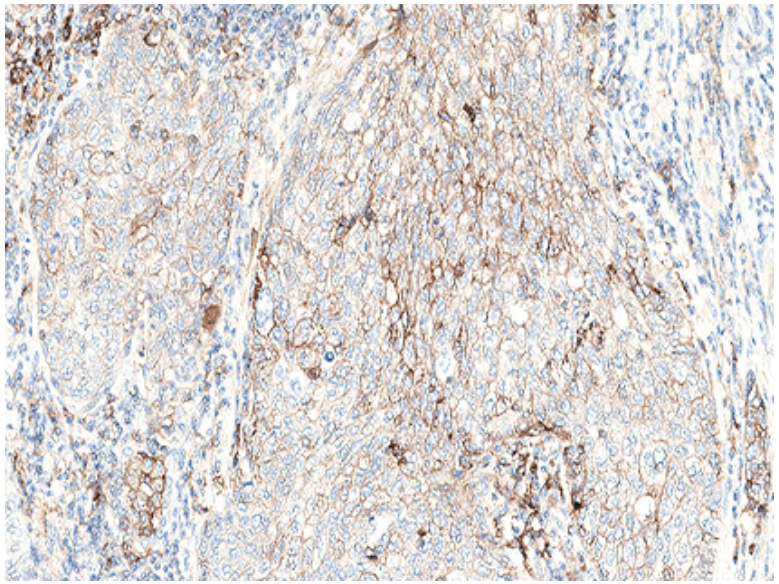
Tonsil

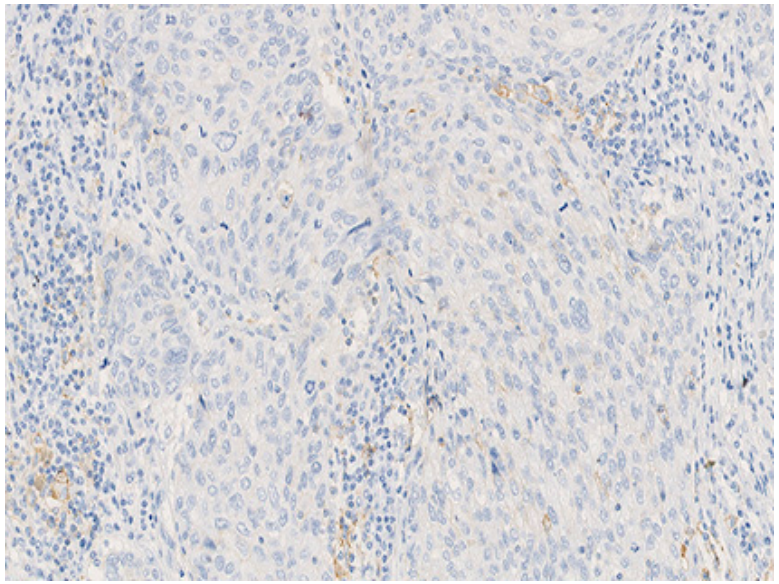




Tonsil

NSCLC
TPS: >50%



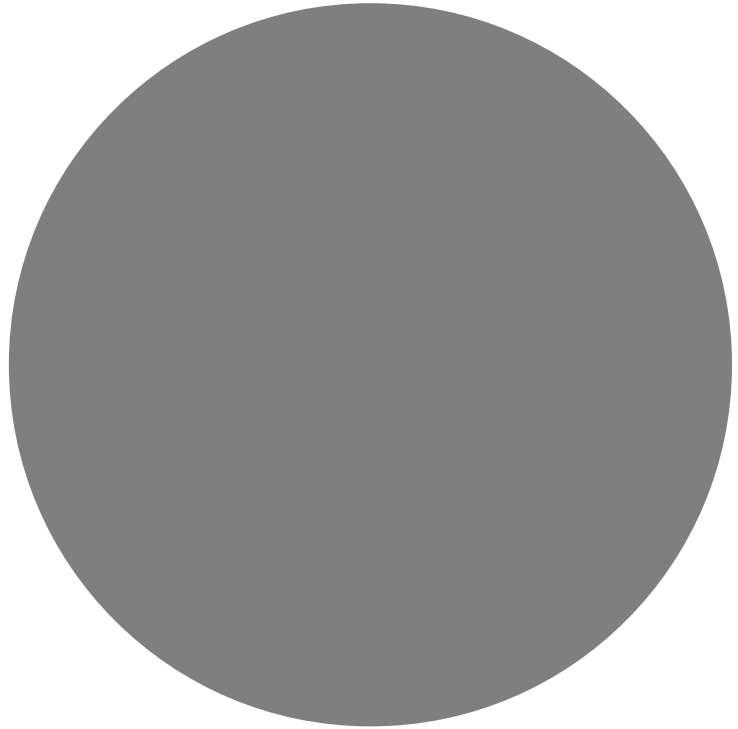


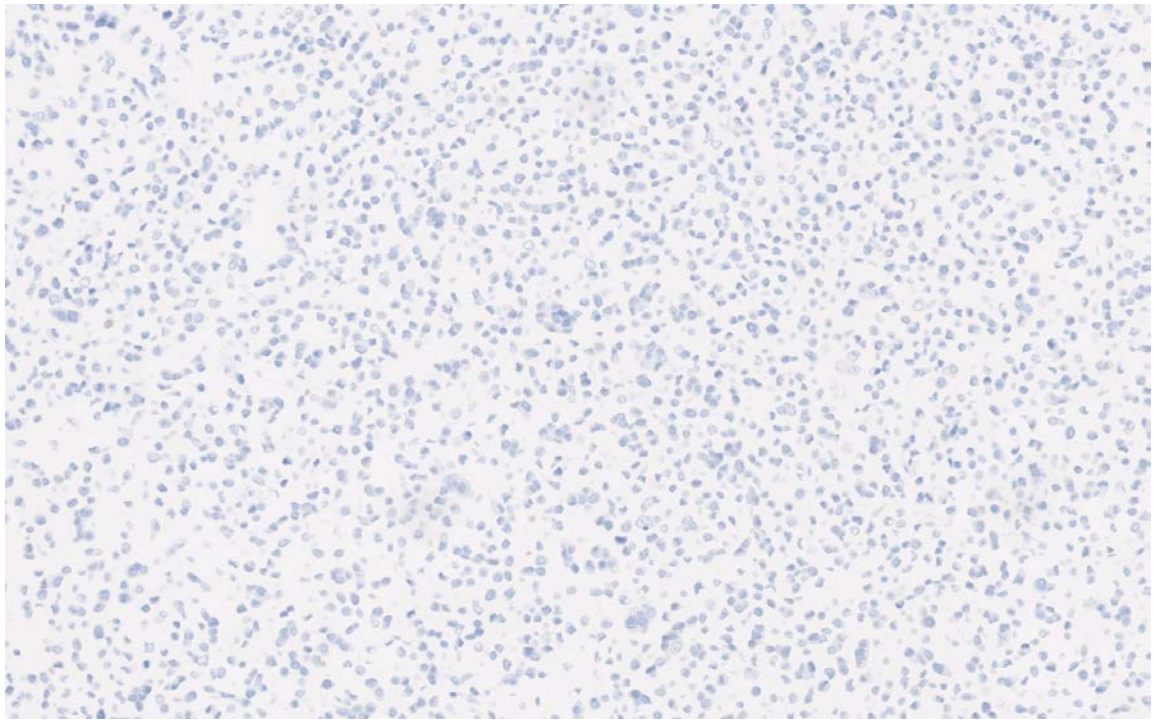
NSCLC
TPS: <50%

NordiQC

Cell lines

PD-L1, C1-C3

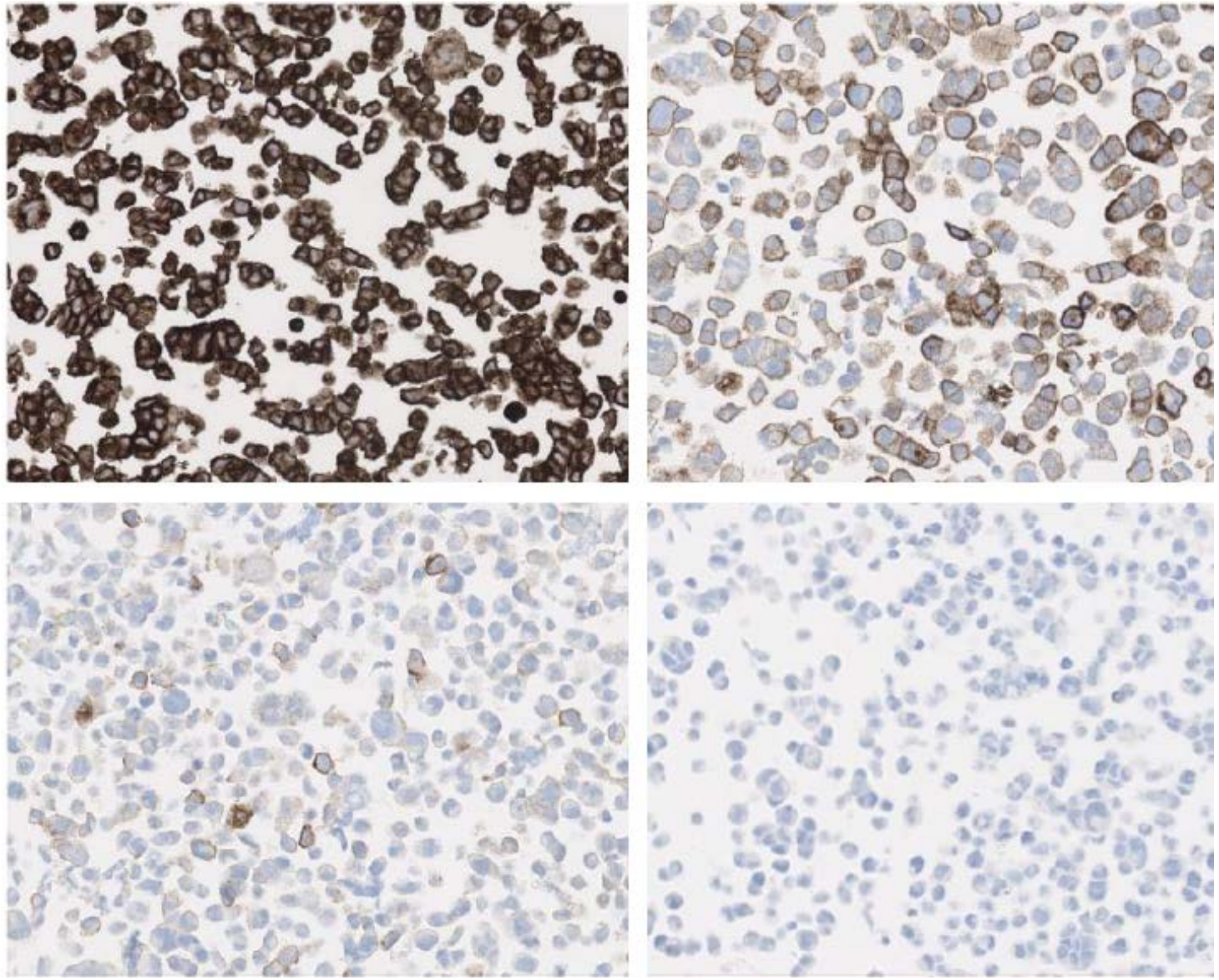




PD-L1 IHC 22C3 pharmDx package controls

PD-L1 Horizon cell lines

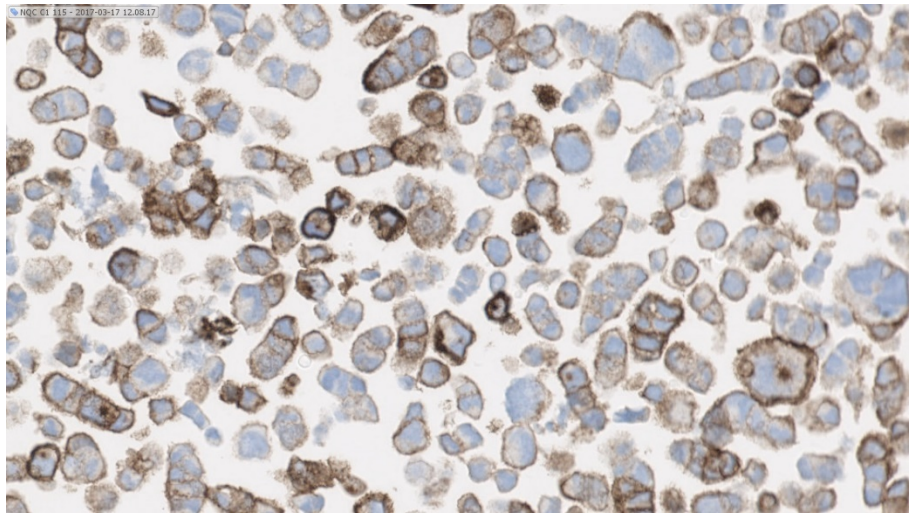
- 1) Strong staining in all cells
- 2) Weak to moderate staining in the majority of cells
- 3) Weak staining in majority of cells
- 4) Negative staining in all cells

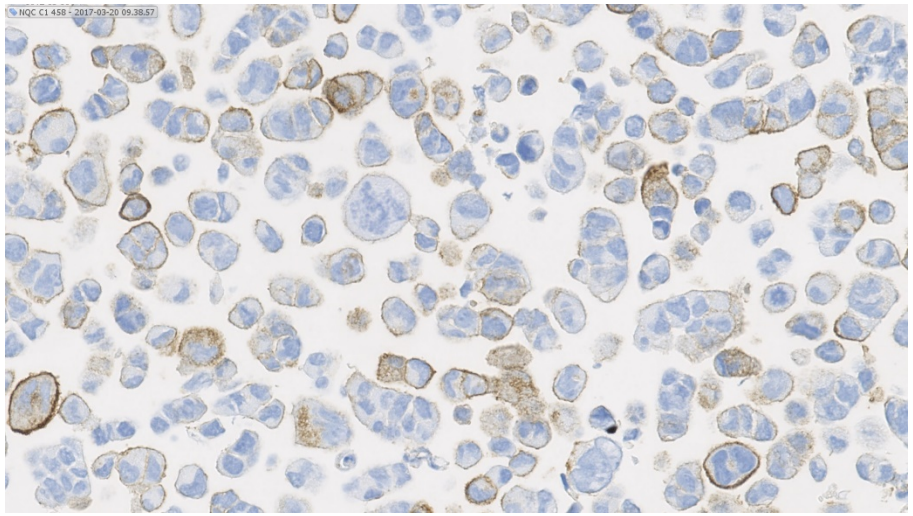


Optimal

Poor

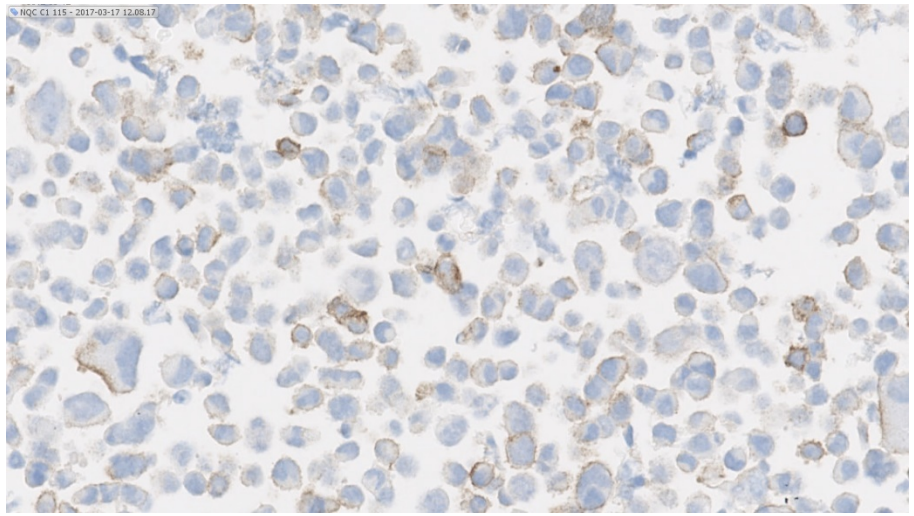
Core 2 (majority weak to moderate stainin)

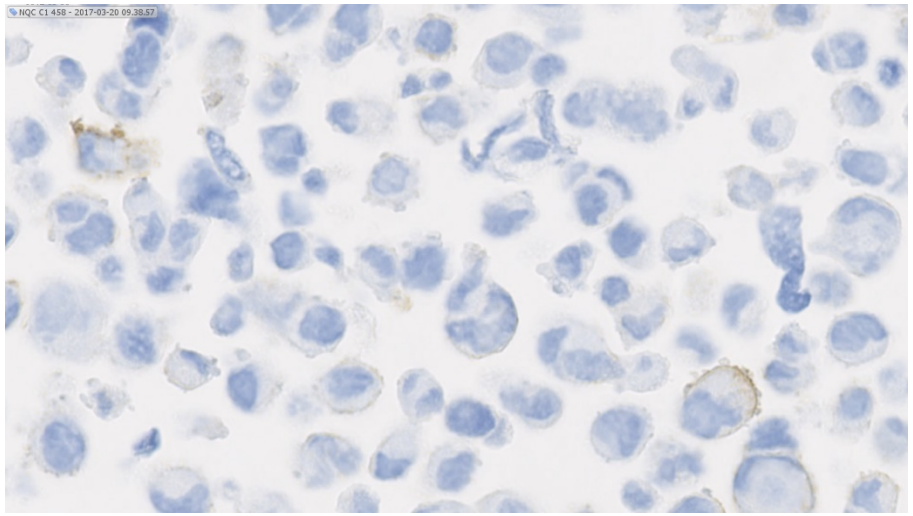




Core 2 (significant loss of cells)

Core 3 (majority, weak staining)



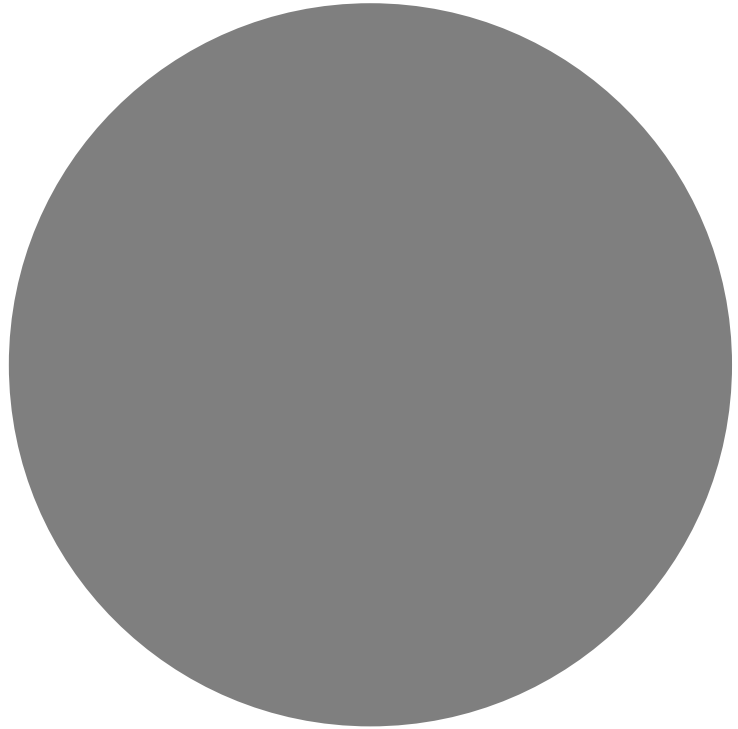


Core 3 (significant loss of cells)

NordiQC

LDT mAb 22C3

Protocols for other platforms



NordiQC

Results C2

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22C3 pharmDX, **SK006**[4]	5	Dako/Agilent	1	2	0	2	60%	-
28-8 pharmDX, **SK005**	6	Dako/Agilent	6	0	0	0	100%	100%
SP263, 790-4905	49	Ventana/Roche	44	2	2	1	94%	98%
SP263, 790-4905[5]	2	Ventana/Roche	0	0	2	0	-	-
Antibodies[3] for laboratory developed PD-L1 assays, conc. antibody	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	39	Dako/Agilent	12	18	4	5	76%	-
mAb clone **E1L3N**	9	Cell Signaling	2	6	1	0	89%	-
mAb **CAL10**	2	Biocare	0	1	0	1	-	-
mAb **CAL10**	1	Zytomed	0	0	0	1	-	-
rmAb clone **28-8**	3	Abcam	1	1	1	0	-	-
rmAb clone **ZR3**	1	Zeta Corporation	1	0	0	0	-	-
rmAb clone **ZR3**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **ZR3**	1	Gene Tech	0	1	0	0	-	-
rmAb clone **SP142**	1	Spring Biosystems	0	0	1	0	-	-
rmAb clone **QR1**	1	Quartett	1	0	0	0	-	-
rmAb clone **HDX3**	1	Halioseek	1	0	0	0	-	-
Total	145		85	38	11	11	-	-
Proportion			59%	26%	8%	8%	85%	-

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.
4) RTU system developed for the Agilent/Dako`s semi-automated systems (Autostainer Link48) but used by laboratories on different platforms (Ventana Benchmark and Dako Omnis).
5) RTU system developed for the Ventana/Roche`s automated systems (BenchMark) but used by laboratories on different platforms (Leica Bond and Dako AS48).

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22C3 – LDT

Accurate PD-L1 Protocols for Non–Small Cell Lung Cancer can be Developed for Automated Staining Platforms With Clone 22C3

Rasmus Røge, MD,† Mogens Vyberg, MD,*† and Søren Nielsen, HT**

Appl Immunohistochem Mol Morphol • Volume 25, Number 6, July 2017

TABLE 1. Protocol Parameters for the PD-L1 IHC 22C3 pharmDx Kit and the Optimized Protocols

Platform	AS48 Dako Link, 22C3 pharmDx, SK006 Dako	Dako Omnis, 22C3 Concentrate, M3653 Dako	BenchMark Ultra, Ventana, 22C3 Concentrate, M3653 Dako	BOND III, Leica, 22C3 Concentrate, M3653 Dako
HIER conditions	20 min at 97°C in target retrieval solution low pH 6.1—off board in PT-Link	40 min at 97°C in target retrieval solution low pH 6.1 GV805, Dako	48 min at 99°C in cell conditioning 1, pH 8.5 950-224, Ventana	30 min at 100°C in epitope retrieval solution 2 pH 9.0 AR9640, Leica
Primary antibody conditions	SK006, ready-to-use Incubation for 30 min at room temperature	M3653, 1:20* Incubation for 40 min at 22°C	M3653, 1:40* Incubation for 64 min at 36°C	M3653, 1:20* Incubation for 60 min at room temperature
Detection system conditions	SK006, ready-to-use Incubation for 30 min in linker and 30 min in polymer at room temperature	GV800/821, Dako Incubation for 30 min in linker and 30 min in polymer at 22°C	760-700, Ventana Incubation for 8 min in linker and 8 min in multimer at 36°C	DS9800, Leica Incubation for 20 min in postblock and 20 min in polymer at room temperature
Chromogen conditions	SK006, ready-to-use Incubation for 2 × 5 min at room temperature	GV825, Dako Incubation for 2 × 5 min at 22°C	760-700, Ventana Incubation for 8 min at 36°C	DS9800, Leica Incubation for 8 min at room temperature

*Diluted in antibody diluent K8006, Dako.
HIER indicates heat-induced epitope retrieval; IHC, immunohistochemistry; PD-L1, programmed death-ligand 1.

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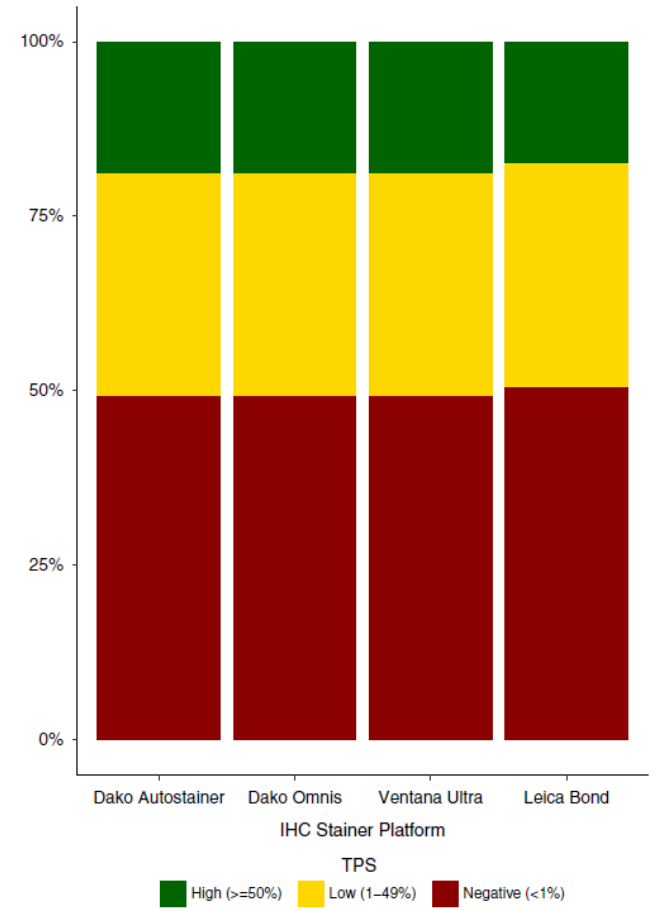
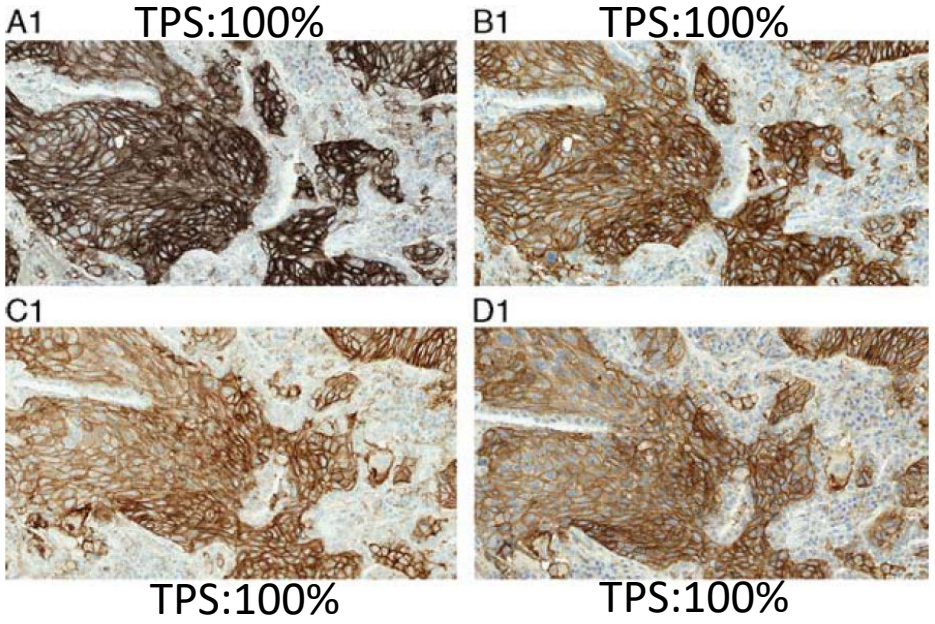
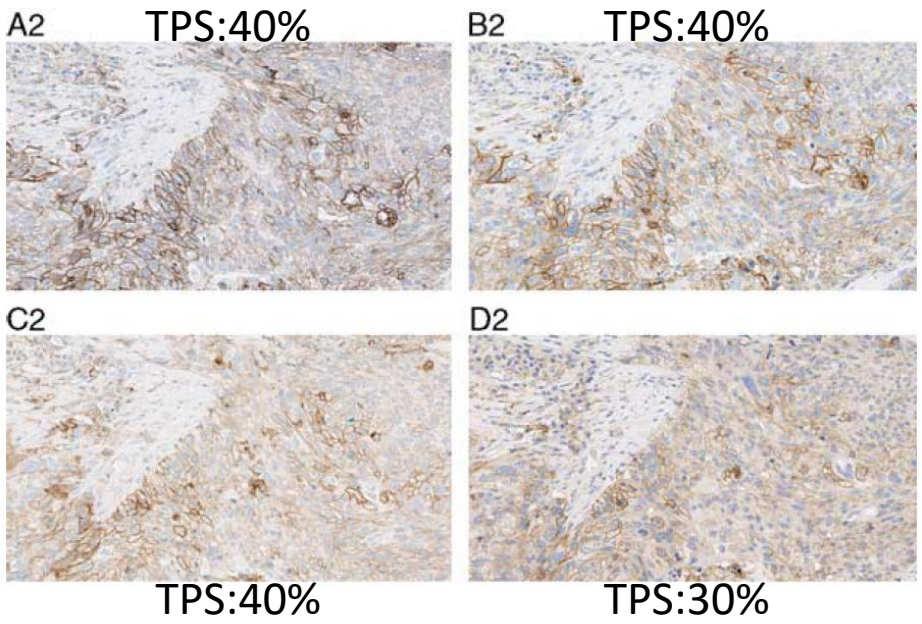
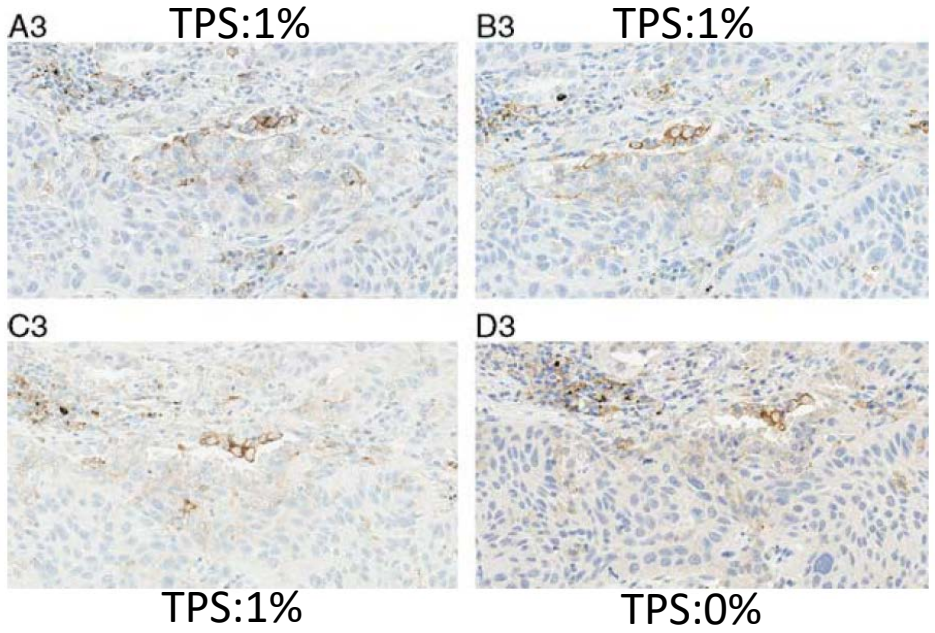


FIGURE 2. Distribution of tumor proportion scoring on the different immunohistochemistry stainer platforms.







Optimized protocols 22C3

A) Dako Autostainer (pharmDx)
B) Dako Omnis
C) Ventana Benchmark
D) Leica Bond

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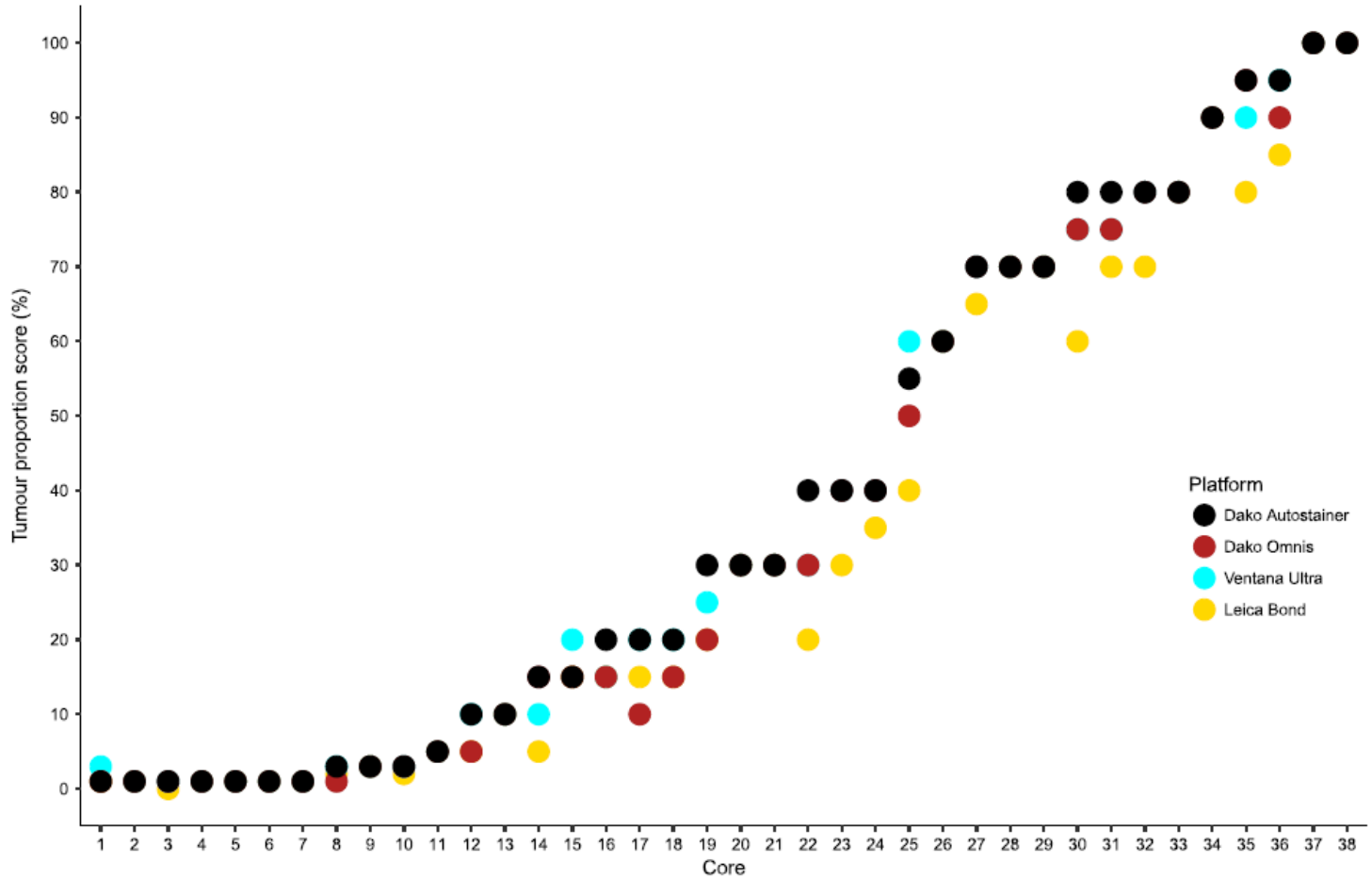

FIGURE 3. Proportion of programmed death-ligand 1 positive tumor cells on the different immunohistochemistry stainer platforms (programmed death-ligand 1 positive tumors only).

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Conclusions

Accurate PD-L1 Protocols for Non–Small Cell Lung Cancer can be Developed for Automated Staining Platforms With Clone 22C3

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- ”Best practice” PD-L1 22C3 protocols identified
- Variations in TPS score between the different stainer platforms
- Overall, Leica Bond platform produced slides with marginally lower TPS
- However, concardance in TPS categories was excellent

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RESEARCH ARTICLE

Use of the 22C3 anti–PD-L1 antibody to determine PD-L1 expression in multiple automated immunohistochemistry platforms

Marius Ilie[1,2,3], Shirin Khambata-Ford[4], Christiane Copie-Bergman[5,6,7], Lingkang Huang[4], Jonathan Juco[4], Veronique Hofman[1,2,3], Paul Hofman[1,2,3]*

PLOS ONE | https://doi.org/10.1371/journal.pone.0183023 August 10, 2017

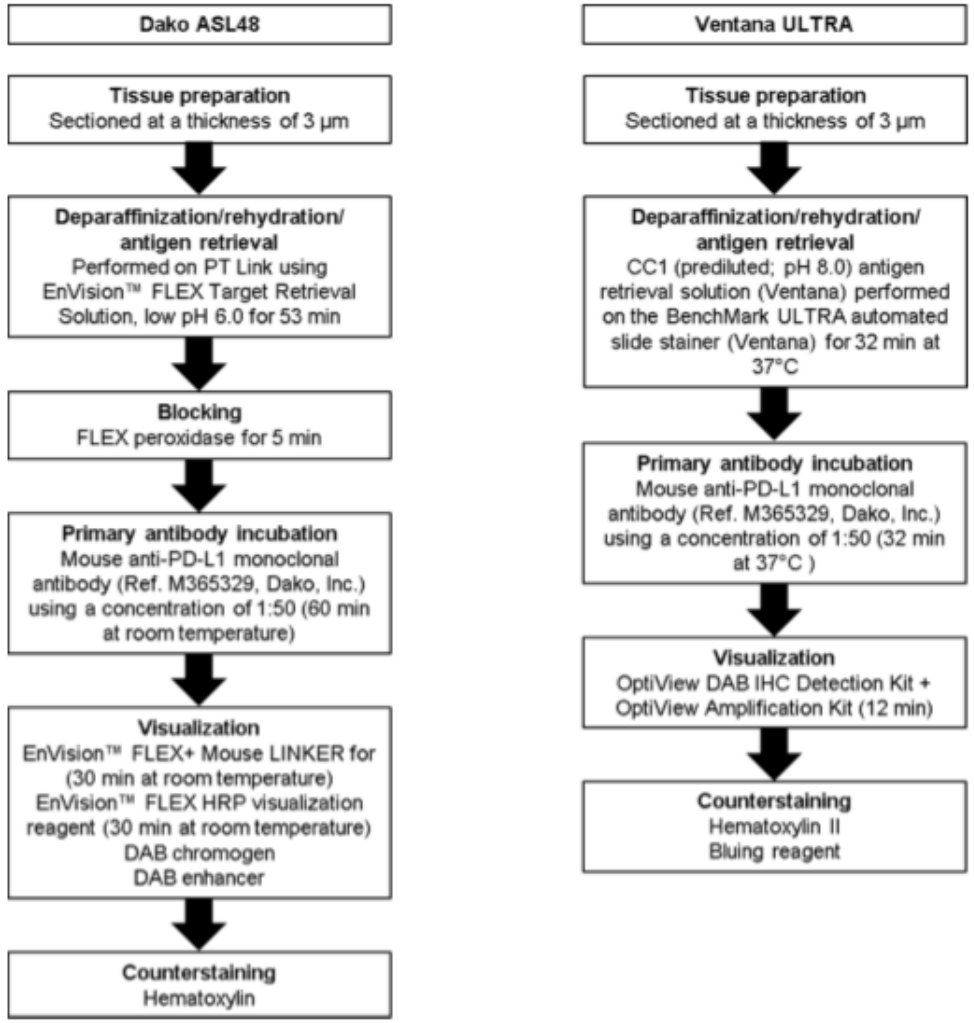

Fig 1. Optimised protocols for PD-L1 IHC assays using the 22C3 antibody concentrate on the Dako ASL48 and VENTANA BenchMark ULTRA platforms. PD-L1, programmed death ligand 1; IHC, immunohistochemistry; ASL48, Autostainer Link 48; DAB, 3,3'-diaminobenzidine tetrahydrochloride.

https://doi.org/10.1371/journal.pone.0183023.g001

Questions?

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