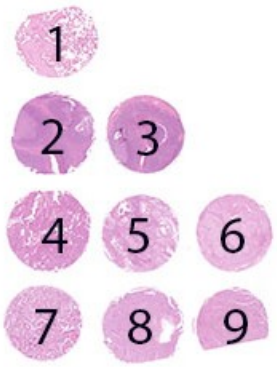
Assessment Run C19 2026
PD-L1 TPS/CPS

Purpose
This was the nineteenth assessment for PD-L1 in the NordiQC Companion Module. This assessment for PD-L1 TPS/CPS primarily focused on evaluating the analytical accuracy of the IHC assays performed by the NordiQC participants to identify patients with non-small cell lung carcinoma (NSCLC) and triple-negative breast carcinoma (TNBC) for treatment with KEYTRUDA® as immunotherapy. PD-L1 22C3 pharmDx (Dako/Agilent) was used as the reference standard method, and accuracy was evaluated in carcinomas with the dynamic and clinically relevant PD-L1 expression levels characterised by TPS and CPS. The scores obtained by NordiQC participants are indicative of the performance of the IHC tests, but due to the limited number and composition of samples, additional internal validation/verification and extended quality control, e.g. regularly measuring the PD-L1 results, is needed.

Material
Table 1. **Content of the TMA used for the NordiQC PD-L1 TPS/CPS (KEYTRUDA®) C19 assessment.**

	PD-L1 IHC TPS/CPS score*
Tissue controls	
1. Placenta	See section for controls
2. Tonsil	See section for controls
3. Tonsil	See section for controls
Carcinomas	
4. NSCLC	TPS: Negative; <1%
5. NSCLC	TPS: High; ≥50%
6. NSCLC	TPS: High; ≥50%*
7. Breast carcinoma	CPS: <10 IC[#]
8. Breast carcinoma	CPS: ≥10; IC[#]
9. Breast carcinoma	CPS: ≥10; IC+TC[#]



*Core 6 was excluded from certain assessments due to the absence of a tumour in a limited number of sections from TMA block.
[#] IC: Immune cells, TC: Tumour cells.

All tissues were fixed in 10% neutral buffered formalin.

The participating laboratories were asked to perform their PD-L1 IHC assay for predicting likely response to KEYTRUDA® as a treatment option, evaluate the PD-L1 expression level using the TPS and CPS scoring system, and to submit their stained slides and scores to NordiQC. This allowed assessment of the technical performance (analytical accuracy) of the PD-L1 TPS/CPS assays and provided information on the reproducibility and concordance of the PD-L1 read-out results among the laboratories.

PD-L1 TPS/CPS, Technical assessment
In order to account for heterogeneity of PD-L1 expression in the individual tumour cores included in the tissue micro array (TMA) blocks, reference slides were prepared throughout the blocks. The PD-L1 expression levels were thus characterised in every twenty-fifth slide, and during the assessment, TPS and CPS categories for each tissue core on the submitted slides from the participants were compared to the level in the nearest reference slide.

Criteria for assessing a staining as <u>Optimal</u> include:
The staining is considered perfect or close to perfect in all of the included tissues.
<u>TPS/CPS is concordant to the NordiQC reference data in all carcinomas.</u>

Criteria for assessing a staining as <u>Good</u> include:
The staining is considered acceptable (correct PD-L1 TPS/CPS category) in all of the included tissues.
PD-L1 expression in one or more tissues varies significantly from the expected TPS/CPS scores, but still in the correct category. The protocol may be optimised to ensure analytical accuracy.
The technical quality may be improved for e.g. counter staining, morphology and signal-to-noise ratio.
<u>TPS/CPS is still concordant to the NordiQC reference data obtained in all carcinomas</u>.

Criteria for assessing a staining as <u>Borderline</u> include:
The staining is considered insufficient because of a false negative or false positive staining reaction in one of the included carcinomas. The protocol should be optimised.
<u>TPS/CPS is **not** concordant to the NordiQC reference data in one of the carcinomas.</u>

Criteria for assessing a staining as <u>Poor</u> include:
The staining is considered very insufficient e.g. because of a false negative or a false positive staining reaction of more than one of the included carcinomas.
Optimisation of the protocol is urgently needed.
<u>TPS/CPS is **not** concordant to the NordiQC reference data in two or more of the carcinomas.</u>

An IHC result can also be assessed as **borderline/poor** related to technical artefacts, e.g. poor signal-to-noise ratio, excessive counterstaining, impaired morphology and/or excessive staining compromising the scoring.

KEY POINTS FOR PD-L1 TPS/CPS IMMUNOASSAYS

- The **CDx** IHC assays with one or more predictive claims provided an overall pass rate of 97% compared to 86% for LD assays.
- The **22C3** CDx assay GE006, Dako/Agilent was most successful with a pass rate of 98% with VRPS, 92% optimal.
- The SK006 assay also showed robust performance, achieving a 100% pass rate with a lower proportion of optimal results at 71%
- Ventana/ Roche **SP263** assays for BenchMark platforms demonstrated high technical robustness, with pass rates of 97-100% and optimal rates of 45-50%.
- Insufficient results were most frequently due to technical deficiencies, including poor signal-to-noise ratio, excessive cytoplasmic staining, and coarse or indistinct granular staining affecting PD-L1 assessment in one or more of the NSCLC and breast carcinoma samples. False-negative results caused by reduced PD-L1 positive cell proportions were the second most common cause.

Participation

Number of laboratories registered for PD-L1 KEYTRUDA IHC C19	335
Number of laboratories returning PD-L1 KEYTRUDA IHC slides	299 (89%)
Number of laboratories returning PD-L1 scoring sheet	265

Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

Results
299 laboratories participated in this assessment and returned slides. 96% of the participants achieved a sufficient mark. Assessment marks for IHC PD-L1 assays and PD-L1 antibodies are summarised in Table 2a-2d (see pages 3-5).

Controls
Throughout all assessments for PD-L1 TPS/CPS, tonsil and placenta have been used as positive and negative tissue controls and tonsil has been found to be superior to placenta, as tonsil typically displays a dynamic and clinically relevant range of PD-L1 expression levels from low to high, whereas placenta typically contains only cells (trophoblasts) with high level PD-L1 expression.
In tonsil, protocols with optimal results for PD-L1 TPS/CPS status typically provide the following reaction pattern:
A moderate to strong predominantly membranous staining reaction in the crypt epithelial cells, a weak to moderate, typically punctated, membranous staining reaction of the majority of germinal centre macrophages and scattered intra- and interfollicular lymphocytes and macrophages showing a coarse punctate granular cytoplasmic staining reaction. No staining reaction in the vast majority of lymphocytes and normal stratified squamous epithelial cells.
It has been observed that different assays and/or clones for PD-L1 TPS/CPS status give different staining patterns in tonsil, which must be taken into account when evaluating the reaction pattern and to verify if the result is as expected. The rmAb clone SP263 (741-4905, 790-4905, 740-4907), Ventana/Roche) typically provide a higher proportion of positive inter- and intra-follicular immune cells compared to the Dako/Agilent 22C3 PD-L1 assays (SK006 and GE006).

For other clones, e.g. rmAb clone CAL10 and E1L3N typically a stronger staining reaction in more germinal centre macrophages were seen compared to mAb clone 22C3, when the clones still provided otherwise optimal and accurate results in the carcinomas. This emphasizes that the expected test performance characteristics in tonsil must be correlated to the PD-L1 IHC test/clone used both for the inter- and intra-PD-L1 IHC reproducibility evaluation.

Conclusion
This was the nineteenth NordiQC assessment of PD-L1 for TPS/CPS status with a focus on NSCLCs and TNBCs. 299 laboratories participated and a pass rate of 96% was observed.

The PD-L1 IHC pharmDx assay based on clone **22C3** (Dako/Agilent) demonstrated the highest overall performance in this assessment. The **GE006** assay, applied according to the vendor-recommended guidelines, achieved a pass rate of 98% and an optimal rate of 92%, representing the best overall performance among the companion diagnostic assays. The SK006 assay also performed very well, providing a pass rate of 100%; however, the proportion of optimal results was lower at 71%. The widely used Ventana/Roche PD-L1 IHC assays **741-4905** and **740-4907** for BenchMark (Ultra/XT/GX) platforms, based on the rmAb clone **SP263**, achieved a pass rate of 97% and 100%, respectively, with optimal rates of 45% and 50%. This represents an improvement compared with the previous run (C18), particularly in the proportion of optimal results, although performance differences between assay systems remained evident.

In this assessment run, the majority of insufficient results were attributed to a technical deficiency, including extensive cytoplasmic staining reactions and poor signal-to-noise ratios, observed in one or more of the NSCLC and TNBC samples. A reduced proportion of PD-L1-positive cells compared to levels expected and defined by the NordiQC reference standard method, leading to false-negative results, was the second most frequent cause of insufficient results. This pattern differs from that observed in C17 and C18, where reduced analytical sensitivity and false negative staining reactions were the predominant causes of assay failure. Instead, the findings in C19 are more comparable to those observed in C15 and C16, where technical deficiencies represented the major source of insufficient results.

Table 2a. **Overall results for PD-L1 TPS/CPS, run C19**

	n	Optimal	Good	Borderline	Poor	Sufficient results[1]	Optimal results
CE-IVD / FDA approved PD-L1 assays*	188	131	52	3	2	97%	70%
Laboratory developed PD-L1 assays based on concentrated antibodies	67	40	26	1	0	99%	60%
PD-L1 assays based on Ready-To-Use antibodies without predictive claims	44	21	17	4	2	86%	48%
Total	299	192	95	8	4		
Proportion		64%	32%	3%	1%	96%	

1) Optimal and good
* Including all protocol settings - both performed as per recommended guidelines or modified settings.

Table 2b. **Assessment marks for CE-IVD / FDA approved PD-L1 assays for PD-L1 TPS/CPS, run C19**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP263, **741-4905 (VRPS)[3]**	60	Ventana/Roche	27	31	2	-	97%	45%
rmAb clone SP263, **741-4905 (LPMS)[4]**	2	Ventana/Roche	2	-	-	-	-	-
rmAb clone SP263, **740-4907 (VRPS)[3]**	14	Ventana/Roche	7	7	-	-	100%	50%
rmAb clone SP142, **741-4860 (VRPS)[3]**	1	Ventana/Roche	-	-	-	1	-	-
mAb clone 22C3 pharmDx, **SK006 (VRPS)[3]**	28	Dako/Agilent	20	8	-	-	100%	71%
mAb clone 22C3 pharmDx, **SK006 (LMPS)[4]**	7	Dako/Agilent	5	2	-	-	100%	71%
mAb clone 22C3 pharmDx, **GE006 (VRPS)[3]**	65	Dako/Agilent	60	4	-	1	98%	92%
mAb clone 22C3 pharmDx, **GE006 (LMPS)[4]**	9	Dako/Agilent	9	-	-	-	100%	100%
rmAb clone 28-8 pharmDx, **SK005 (VRPS)[3]**	1	Dako/Agilent	-	-	1	-	-	-
rmAb clone 28-8 pharmDx, **SK005 (LMPS)[4]**	1	Dako/Agilent	1	-	-	-	-	-
Total	188		131	52	3	2		
Proportion			70%	27%	2%	1%	97%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.
4) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.

Table 2c. **Assessment marks for concentrated antibodies for PD-L1 TPS/CPS, run C19**

Antibodies[5] for laboratory developed PD-L1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **22C3**	58	Dako/Agilent	37	20	1	-	98%	63%
rmAb clone **QR001**	4	Quartett	2	2	-	-	-	-
rmAb clone **E1L3N**	3	Cell Signaling	-	3	-	-	-	-
rmAb **CAL10**	2	Biocare Medical Zytomed	1	1	-	-	-	-
Total	67		40	26	1	0		
Proportion			60%	39%	1%	0%	99%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
5) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.

Table 2d. **Assessment marks for Ready-To-Use antibodies[6] for PD-L1 TPS/CPS, run C19**

Ready-To-Use antibodies[6]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone SP263, **790-4905 (VRPS)[3]**	13	Ventana/Roche	3	9	1	-	92%	23%
rmAb clone SP263, **790-4905 (LMPS)[4]**	16	Ventana/Roche	10	6	-	-	100%	63%
rmAb clone SP263, **741-7139 (LMPS)[4]**	1	Ventana/Roche	1	-	-	-	-	-
rmAb clone SP142, **790-4860 (LMPS)[4]**	1	Ventana/Roche	-	-	-	1	-	-
rmAb clone 73-10 **PA0832**	3	Leica Biosystems	1	2	-	-	-	-
rmAb clone MX070C **MAB-0854**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone BP6099 **I12052E**	1	Biolynx	1	-	-	-	-	-
rmAb clone CAL10 **PA045-RUO**	1	Leica Biosystems	1	-	-	-	-	-
rmAb clone QR001 **P-P001-30/70/150**	5	Quartett	2	-	2	1	-	-
rmAb Clone DGM274 **RBG081**	1	IntelliPATH	-	-	1	-	-	-
rmAb Clone DY49318 **4023242**	1	ImmunoLogic	1	-	-	-	-	-
Total	44		21	17	4	2	-	
Proportion			48%	38%	9%	5%	86%	

1) Proportion of sufficient results (optimal or good).
2) Proportion of optimal results.
3) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.
4) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.
6) Ready-To-Use antibodies without predictive claim.

Detailed Analysis

CE IVD / FDA approved assays

rmAb clone **SP263** (741-4905, Ventana/Roche): In total, 27 of 60 (45%) protocols were assessed as optimal. This product has a locked protocol on all BenchMark platforms and cannot be changed. The protocol is based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1) at 100°C for 64 min., 16 min. incubation of primary Ab and OptiView as detection system. Using these protocols settings and applied on the BenchMark platform, 58 of 60 (97%) laboratories produced a sufficient staining result (optimal or good).
The product was used by 2 laboratories on a non-intended platform. This data is not included.

rmAb clone **SP263** (740-4907, Ventana/Roche): In total, 7 of 14 (50%) protocols were assessed as optimal. This product has a locked protocol on the BenchMark platform and cannot be changed. The protocol is based on HIER in CC1 at 100°C for 64 min., 16 min. incubation of primary Ab and OptiView as detection system. Using these protocols settings, 14 of 14 (100%) laboratories produced a sufficient staining result.

PD-L1 IHC 22C3 pharmDx (SK006, Dako/Agilent): In total, 20 of 28 (71%) protocols were assessed as optimal, when applied accordingly to the vendor recommended protocol settings based on HIER using EnVision™ FLEX Target Retrieval Solution (TRS) low pH 6.1 at 95-99°C for 20 min. in PT Link, 30 min. incubation of the primary antibody, EnVision™ FLEX+ as the detection system and performed on Autostainer Link 48. Using these protocol settings, 28 of 28 (100%) laboratories produced a sufficient staining result.
SK006 was also used with modified protocol settings, e.g., minor changes in HIER time and incubation time of primary Ab.
The product was used by 2 laboratories on a non-intended platform. This data is not included.

PD-L1 IHC 22C3 pharmDx (GE006, Dako/Agilent): In total, 60 of 65 (92%) protocols were assessed as optimal, when applied according to the vendor-recommended protocol settings HIER using EnVision™ FLEX TRS low pH 6.1 (GV805) at 95-99°C for 40 min., 40 min. incubation of the primary antibody, EnVision™ FLEX+ as the detection system and performed on Omnis. Using these protocol settings, 64 of 65 (98%) laboratories produced a sufficient staining result.

Table 3 summarises the proportion of sufficient and optimal marks for the most commonly used CE IVD / FDA-approved assays. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory-modified systems changing basal protocol settings. Only protocols performed on the intended automated IHC platform are included (in Table 1, LMPS also includes off-label use on deviant IHC stainers).

Table 3. **Comparison of pass rates for vendor recommended and laboratory modified protocols**

CDx assay*	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana BenchMark XT, GX, Ultra rmAb SP263, **741-4905**	58/60 (97%)	27/60 (45%)	-	-
Ventana BenchMark Ultra rmAb SP263, **740-4907**	14/14 (100%)	7/14 (50%)	-	-
Dako Autostainer Link 48+ mAb 22C3 pharmDx, **SK006**	28/28 (100%)	20/28 (71%)	5/5 (100%)	4/5 (80%)
Dako Omnis mAb 22C3 pharmDx, **GE006**	64/65 (98%)	60/65 (92%)	9/9 (100%)	9/9 (100%)
Dako Autostainer Link 48+ rmAb 28-8 pharmDx, **SK005**	0/1	0/1	-	-

*Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
**Modifications in one or more of above-mentioned parameters. Only protocols performed on the specified vendor IHC stainer are included.

Concentrated antibodies for laboratory developed (LD) assays
mAb clone **22C3**: In total, 37 of 58 (64%) protocols were assessed as optimal. Of the 58 protocols, 35 were performed on the BenchMark Ultra platform (Ventana/Roche), 11 on BenchMark Ultra Plus (Ventana/Roche), 6 on Omnis (Dako/Agilent), 4 on Bond III (Leica Biosystems) and 2 on Autostainer Link 48 (Dako/Agilent).

On BenchMark Ultra and Ultra Plus, the protocols providing optimal results were based on a titre of 1:20-50 for mAb clone 22C3, incubation time of 24-92 min., HIER in CC1 for 48-80 min. and OptiView as the detection system. Using these protocol settings, 29 of 46 (63%) laboratories produced optimal staining results, and 46 of 46 (100%) laboratories produced sufficient staining results.
On Omnis, the protocols providing optimal results for mAb clone 22C3 were based on a titre of 1:20-50 of the primary antibody, incubation time of 30-40 min., HIER in TRS low pH 6.1 at 97°C for 30-50 min. and EnVision™ FLEX+ as detection system. Using these protocol settings, 5 of 6 (83%) laboratories produced optimal results and 6 of 6 (100%) laboratories produced a sufficient staining result.

rmAb clone **QR001**: 2 of 4 protocols were assessed as optimal. The optimal protocols for this clone were based on a titre of 1:100 of the primary antibody, incubation time of 15 min., HIER in Bond Epitope Retrieval Solution (BERS2, Leica Biosystems) pH 9 at 100°C for 20 min., Bond™ Refine was applied as the detection system and performed on Bond III (3/4) & MAX (1/4) platforms (Leica Biosystems). Using these protocol settings, 2 of 4 (50%) laboratories achieved optimal results, and 4 of 4 (100%) laboratories achieved sufficient staining.

Table 4. **Proportion of optimal results for PD-L1 on the four main IHC systems by optimal titration settings as listed above**

Concentrated antibodies	**Ventana/Roche BenchMark [1]**		**Dako/Agilent Omnis**		**Dako/Agilent Autostainer [2]**		**Leica Biosystems Bond[3]**	
	CC1 pH 8.5	CC2 pH 6.0	TRS pH 9.0	TRS pH 6.1	TRS High pH	TRS Low pH	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **22C3**	29/46* (63%)	-	-	1/2*	-	5/6* (83%)	2/3*	0/1*

*number of optimal results/number of laboratories using this buffer.
1) BenchMark Ultra, Ultra Plus
2) Autostainer Link 48
3) Bond III

Block construction and assessment reference standards
The tissue micro array (TMA) blocks constructed for this PD-L1 run consisted of three NSCLCs, three breast cancers (BC), two tonsils and one placenta. The NSCLCs were selected to comprise PD-L1 expression levels for each TPS category: TPS negative (<1% PD-L1 positive tumour cells), TPS low (≥1-49%) and TPS high (≥50%). However, due to tissue availability constraints, no core with low TPS expression but two cores with high TPS expression were included, selected to exhibit differing staining intensities to maintain analytical challenge across assays with varying sensitivity. The BCs were selected to comprise one carcinoma with CPS<10 and two carcinomas with CPS≥10, one with PD-L1 expression primarily in immune cells and one with PD-L1 expression in both tumour cells and immune cells. Reference slides throughout the individual

TMA blocks (interval at each twenty-fifth slide) were stained using the companion diagnostic assay 22C3 pharmDx GE006 (Dako/Agilent).

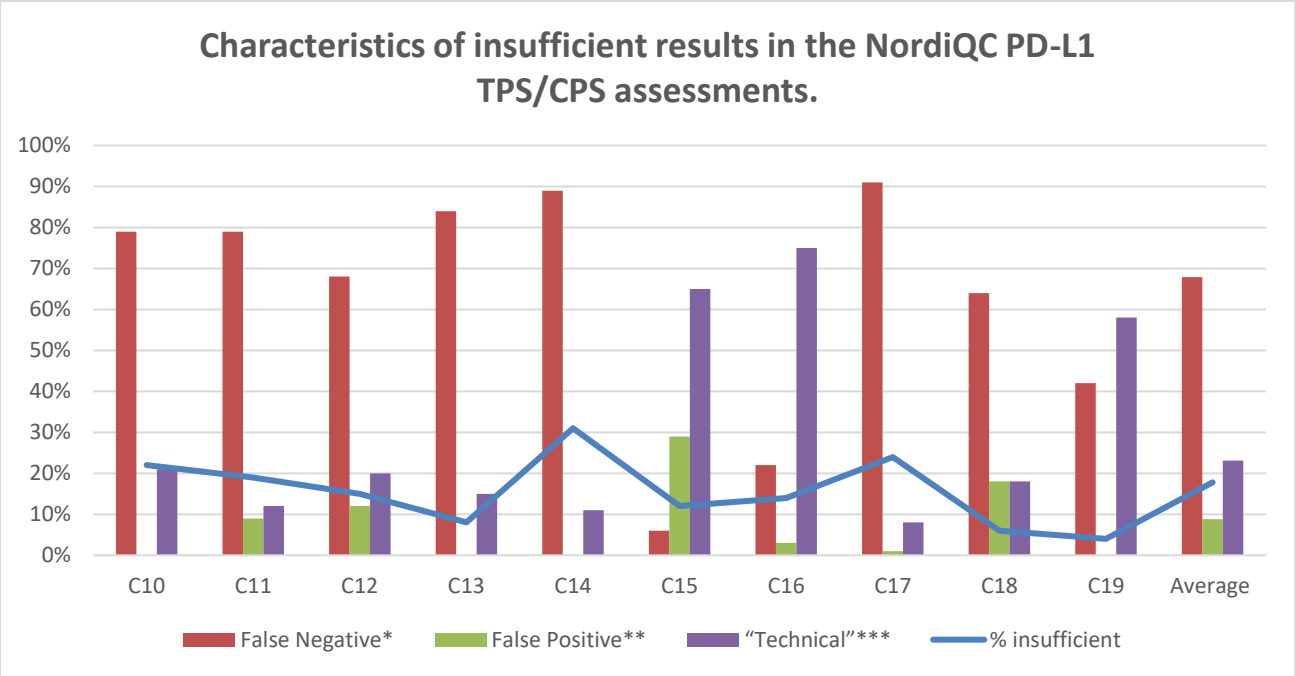
In total, nine identical TMA blocks were constructed, and all nine of these were used for this assessment. After reviewing the reference slides from the blocks, Core 6 was excluded from assessment in slides (n=8) where no tumour tissue was present in the corresponding sections, affecting a subset of participants who received those levels from a limited number of sections from one TMA block. It was also noted that tumour cells were staining in some sections of Core 9, contributing to variability in scoring between adjacent levels. During the assessment, TPS and CPS categories for each tissue core on the submitted slides were compared to the level in the nearest reference slides. Heterogeneity in PD-L1 expression is well known in NSCLCs, and the assessment in this sense emulates clinical settings.

Comments

In this nineteenth NordiQC assessment for PD-L1 TPS/CPS, the prevalent feature of an insufficient staining result was a reduced proportion of PD-L1 positive cells compared to levels expected and defined by the NordiQC reference standard methods, leading to false negative results, being observed in 42% of the insufficient results. None of the insufficient results were caused by false positive staining results and 58% occurred due to technical issues such as poor-signal-to-noise ratio, excessive cytoplasmic staining reaction or a coarse and indistinct granular staining reaction compromising the scoring of the PD-L1 status in one or more of the carcinomas. As shown in Graph. 1, a false negative staining result has been the most common reason for insufficient staining results up until C15, and then again in run C17 and C18. In contrast, technical deficiencies were the most frequent cause of insufficient results in the current run.

In this run, no insufficient results were attributed to false positives, in contrast to C18, where 18% of insufficient results were false positives. While zero false positive results have been observed in several previous runs, the overall average false positive rate across the module is 9%. This may in part reflect the composition of the TMA, which did not include a TPS Low core, a clinically critical threshold where both false positive and false negative errors are most likely to be identified.

Graph 1. **Prevalence and characteristics of insufficient results**



* TPS changes from high to low or low to negative. And/or CPS changes from ≥10 to <10.
** TPS changes from negative to low or low to high. And/or CPS changes from <10 to ≥10.
*** Interpretation compromised e.g. by poor-signal-to noise ratio, poor morphology, excessive cytoplasmic staining reaction etc.

In order to evaluate IHC accuracy, NordiQC strives to include neoplasms with PD-L1 levels close to the critical and clinically relevant thresholds for positivity, focusing on both intensity, proportion and subtypes of cells to be scored, mimicking real-life diagnostics.

The NSCLC, tissue core no. 5, characterised as TPS high by the NordiQC reference standard method, was the most challenging to obtain an optimal result.

32% (n=95) of the slides submitted were assessed as "Good". Of these, 54% of these (52 of 95) were classified as such due to weaker-than-expected staining compared to the reference slides. A further 35% (33 of 95) showed a significantly reduced TPS/CPS level; however, there was no change in the TPS/CPS-category in any of the carcinomas and thus still an accurate PD-L1 status for treatment decision. The remaining 11% (10 of 95) of the 'Good' results were characterised by poor signal-to-noise ratio, too weak or excessive counterstaining and/or a coarse granular staining reaction compromising the evaluation of the membranous staining reaction. The latter only seen for protocols based on OptiView with amplification kit (Ventana/Roche).

The Ventana/Roche PD-L1 IHC assays **741-4905** and **740-4907** for BenchMark (Ultra/XT/GX) with predictive claims, based on the **SP263** clone, were used by 25% (74 of 299) of the participants and in total provided an overall pass rate of 97% (72 of 74), with 46% (34 of 74) being assessed as optimal when applied by protocol settings in compliance with vendor recommendations (see Table 3). The assays are locked for central protocol settings and based on HIER in CC1 for 64 min., incubation in primary Ab for 16 min. and use of OptiView as the detection system. The proportion of optimal results seen are still inferior to the level seen for the 22C3 IHC pharmDx assays (Dako/Agilent), but the gap has significantly improved in the recent runs and this run – C19. In previous assessment runs - C10 onwards, a relatively high number of SP263 results have been characterised by a reduced analytical sensitivity, providing a lower TPS level compared to the level seen for the 22C3 pharmDx assays, with no explanation for this discrepancy having been identified.

The Dako/Agilent **22C3** pharmDx assay **GE006** for Dako Omnis was used by 22% (65 of 299) of the participants, providing a pass rate of 98% (64 of 65) with an optimal rate of 92% (60 of 65) when applied by protocol settings in compliance with vendor recommendations (see Table 3).

Consistent with findings from previous runs, it was observed that the PD-L1 22C3 GE006 assay for Omnis was more successful compared to the **22C3** pharmDx SK006 for Autostainer Link 48. The superior performance of GE006 might in part be related to a more consistent reproducibility of the 22C3 pharmDx assay on the fully automated Dako Omnis platform compared to the assay when applied on the semi-automated Autostainer Link 48. In this context, it should be emphasised that the 22C3 GE006 assay for Dako Omnis was originally validated for NSCLC using TPS as the scoring system. However, the assay has subsequently received FDA approval for additional indications requiring CPS scoring, including TNBC, Cervical cancer, ESCC and gastric/GEJ adenocarcinoma.

The Dako/Agilent **22C3** pharmDx assay **SK006** for Autostainer Link 48 was used by 9% (28 of 299) of the participants and provided a pass rate of 100% (28 of 28) with 71% (20 of 28) being assessed as optimal when applied by protocol settings in compliance with vendor recommendations (see Table 3). The 22C3 SK006 assay was also applied off-label (n=7), both on Autostainer Link 48 using modified protocol settings or on platforms other than the Autostainer Link 48, such as Omnis (Dako/Agilent), and as shown in Table 2b in this run with a pass rate of 100% (7 of 7) with 71% (5 of 7) being assessed as optimal.

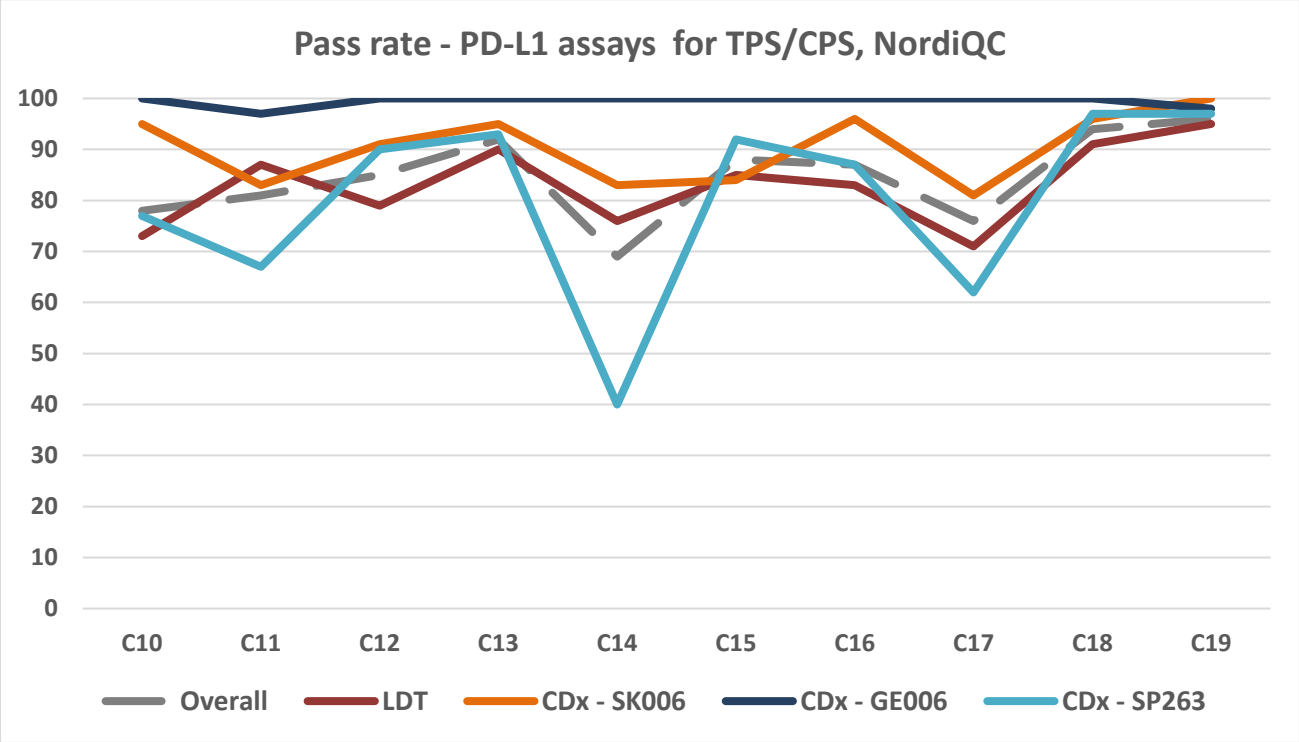
The Dako/Agilent pharmDx **SK005** rmAb **28-8** for Autostainer Link 48 was used by 1 laboratory, using the recommended protocol settings being assessed as borderline. It was applied off-label by 1 laboratory using modified protocol settings on the Omnis being assessed as Optimal.

Overall, 168 participants used one of the PD-L1 IHC CDx assays with one or more predictive claims for immune-oncology (22C3 SK006/GE006, SP263 741-4905/740-4907 and 28-8, SK005) by VRPS and a pass rate of 98% (164 of 168), was achieved.

Laboratory developed (LD) assays either based on a concentrated antibody, a RTU antibody without any predictive claim or a companion diagnostic assay not used strictly accordingly to vendor recommendations were applied by 39% (117 of 299) of the participants. For this group a pass rate 95% was observed which is considerably higher compared to the level of 90% seen in C18 and 71% in C17. Focusing on the performance of PD-L1 LD assays from C2-C19, excluding the initial run C1 and start-up phase to identify "best practice LD assays", the mean pass rate for LD assays has been 80% (range 66%-95%) compared to e.g., 100% for the 22C3 GE006 pharmDx (Dako/Agilent), 89% for 22C3 SK006 pharmDx (Dako/Agilent) and 86% for the SP263 assay (Ventana/Roche).

The performance of CDx and LD IHC assays for PD-L1 is summarised and shown in Graph 2 (page 9).

Graph 2. **Proportion of pass rates for PD-L1 TPS/CPS IHC assays in the NordiC latest 10 runs performed.**



The mAb clone **22C3** was the most widely used concentrated antibody within a LD assay (n=58), achieving a pass rate of 98% (57 of 58) and an optimal rate of 63% (37 of 58). This represents the highest performance observed to date for LD assays and a substantial improvement compared with the C17, in which a pass rate of 76% and an optimal rate of 24% were achieved. The results also extend the positive trend observed in C18, where the pass rate increased to 92% and the optimal rate of 47%.

As described above for optimal protocol settings for mAb clone **22C3** as concentrated format, successful and interlaboratory reproducible settings have been identified for BenchMark (Ventana/Roche) and Omnis (Dako/Agilent) and these seem to be widely consolidated within the laboratories providing a pass rate largely comparable to most companion diagnostic assays in this assessment as shown in Graph 2.

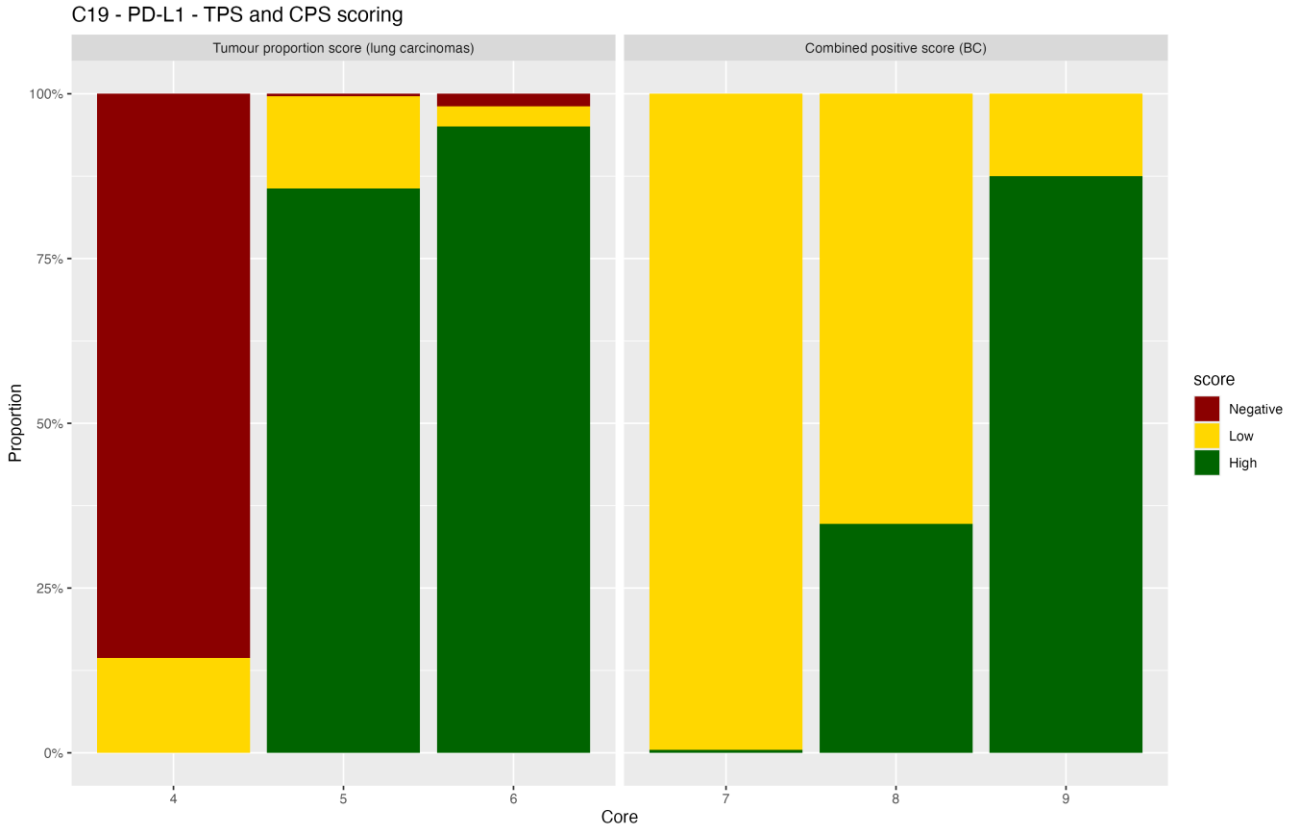
As noted in previous reports, the performance of the mAb clone 22C3 on the Bond III / Bond MAX (Leica Biosystems) platforms has generally been inferior. However, in run C13, a 100% sufficient pass rate was observed, with one optimal result. This improvement was not sustained in subsequent runs C14 and C15, where no participants achieved an optimal result. In C16, one optimal result again was achieved, but this was not maintained in run C17, where no participants achieved sufficient result. In C18, three of the five participants achieved a 'Good' result, while two were assessed as insufficient. In this run C19, four participants used 22C3 on the Leica Bond III platform, with two achieving an optimal result, one good result, and one a borderline result. Cumulated data for runs C8 - C19 evaluating the performance of mAb clone 22C3 on the Bond platforms have shown a pass rate of 42% (20 of 48), with 4 optimal results achieved to date. Given the limited number of observations, these findings should be interpreted with caution.
However, in this context it should be mentioned that the rmAb clone **QR001** (Quartett) seemed to be a more reproducible antibody for the Leica Bond IHC platforms, as 3 out of 3 protocols gave an optimal result when applied by the protocol settings listed on page 6 in C17. In C18, a slightly inferior performance was obtained as only 1 of 3 submitted protocols gave an optimal result, while the remaining achieved a result assessed as good. In the current run (C19), four participants used this protocol, of which two achieved an optimal result, and two achieved a good result. Overall, all ten QR001-based protocols across these three consecutive runs have produced sufficient results, indicating promising reproducibility.

The Leica Biosystems PD-L1 IHC RTU assay, based on rmAb clone **73-10** (**PA0832**) with intended use on Bond III, was used by 3 participants and all 3 results were assessed as sufficient, 1 being optimal. From Run C13 to this run, C19, an overall pass rate of 90% (26/29) has been achieved, and 38% being optimal.

The commonly used Ventana/Roche IHC RTU assay **790-4905**, rmAb clone **SP263** without predictive claim showed a strong performance in run C19. When applied according to vendor-recommended protocol settings, a pass rate of 92% (12 of 13) was achieved, with 23% (3 of 13) assessed as optimal. When used by

laboratory modified protocol settings, all 16 achieved a sufficient result (100%), with 63% (10 of 16) assessed as optimal. No single modified protocol setting, such as prolonging HIER or Ab incubation time, explaining the improved performance could be identified.

PD-L1 interpretation and scoring consensus:
Participants were asked to score each of the cores using either tumour proportion score (TPS) for the NSCLCs or combined positive score (CPS) for the BCs.



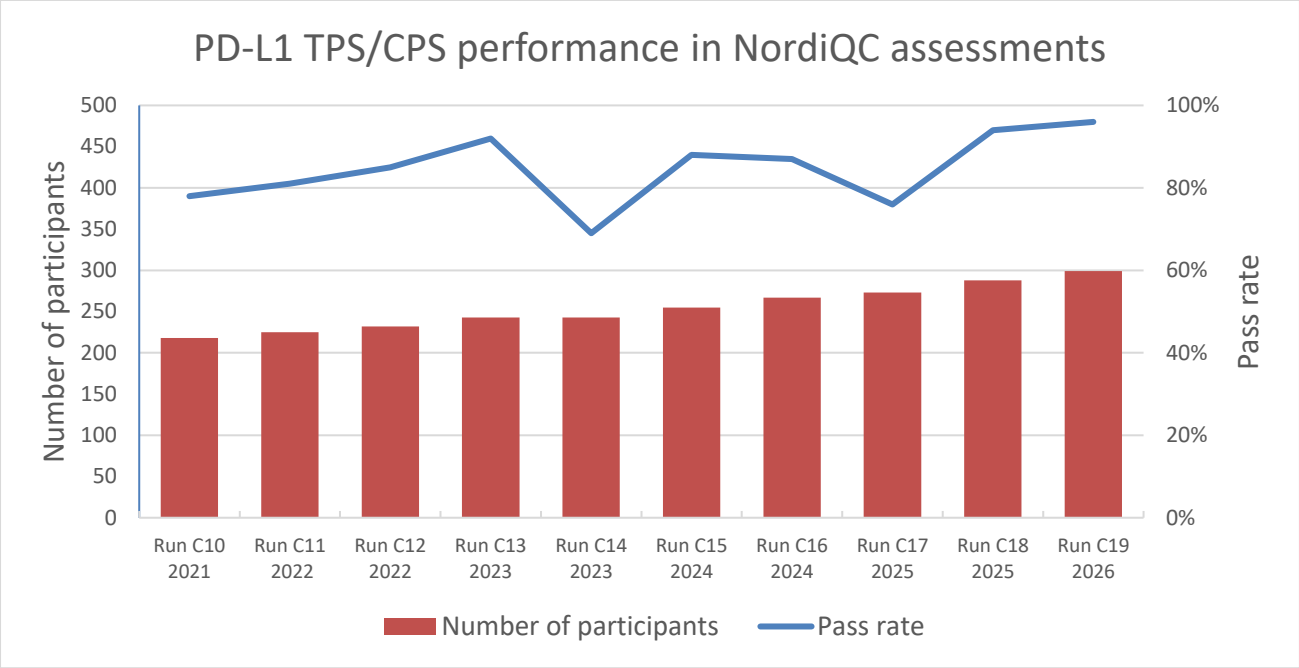
Graph 3. **NordiQC PD-L1 run C19: Tumour Proportion scores (TPS) in NSCLCs (core no. 4-6) and Combined Positive Score (CPS) in BCs (core no. 7-9).**

As shown in Graph 3, relatively high consensus rates were observed for tissue cores 6,7 and 9. In contrast, cores 4,5 and 8 showed lower consensus. The NSCLC, tissue core 4 was by the NordiQC reference method characterized as TPS negative, but contained many immune cells, especially large macrophages, that might have been scored as tumour cells. Tissue core 5 was characterized as TPS high by NordiQC. Tumour cells were weakly stained and was a challenge to score score. Within the BC tissue core 8, immune cells were positive while tumour cells were negative. Moreover, the CPS in many blocks were near the CPS 10 threshold that distinguishes high from low expression, likely contributing to the disagreement observed in core 8.

Performance history
A relatively consistent pass rate has been observed across the module runs, with an overall upward trend, except for C14. The rate remained stable during C15 and C16, declined in C17, increased in C18, and rose slightly again in this run (C19), which achieved the highest pass rate to date, as shown in Graph 4 (see page 10).

The number of new participants has consistently increased by approximately 3-5% in each run. However, it remained unchanged for C13 and C14, before rising again in the most recent runs, C17, C18, and C19, reaching a total of 299 participants and 335 registered. Module membership is currently frozen, and no new participants are able to join at present.

Graph 4. **Proportion of sufficient results for PD-L1 TPS/CPS (KEYTRUDA®) in the latest 10 NordiQC runs performed.**



Summary
The overall pass rate increased to 96% in C19, the highest rate achieved in the NordiQC PD-L1 TPS/CPS module to date. This improvement reflected strong performance by the widely used 22C3 pharmDx GE006 and SK006 assays, improved performance by the SP263 assays and the highest performance recorded to date for laboratory-developed assays based on concentrated mAb clone 22C3. These findings suggest that participating laboratories are increasingly implementing robust and reproducible protocol settings.

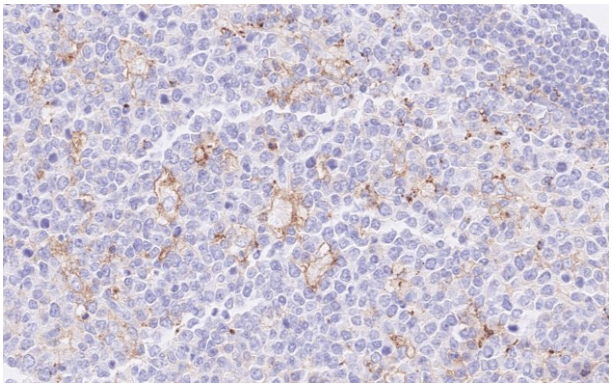


Fig. 1a
Optimal staining result of tonsil using the PD-L1 IHC 22C3 GE006 pharmDx kit, Dako/Agilent, following the vendor recommended protocol settings.
A weak to moderate, but distinct punctuated membranous staining reaction of germinal centre macrophages and dispersed lymphocytes is seen.
No staining reaction is seen in the vast majority of lymphocytes.
Also compare with Figs. 2a – 5a, same protocol.

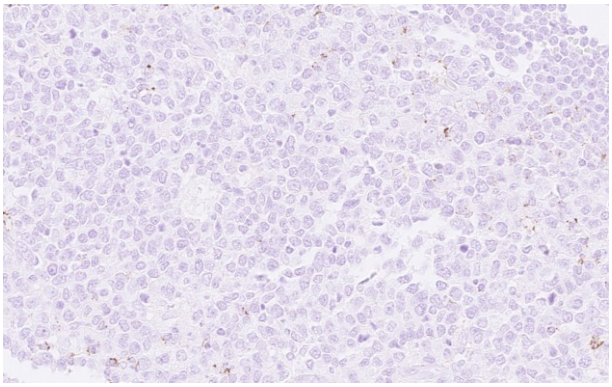


Fig. 1b
Staining result of tonsil, using the rmAb clone QR001 in a prediluted format on a Ventana BenchMark platform.
Scattered T-cells are demonstrated showing a granular punctuated staining reaction. The germinal centre macrophages are negative.
Also compare with Figs. 2b - 4b, same protocol.

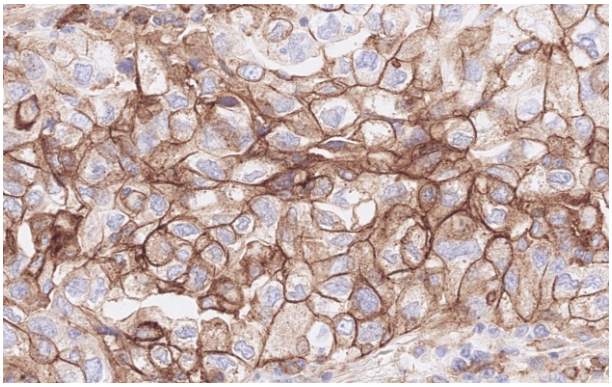


Fig. 2a
Optimal staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1a.
A moderate to strong, distinct membranous staining reaction is seen in about the majority of tumour cells.
Overall, the tumour was categorized as TPS High (≥50%) and thus eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur).

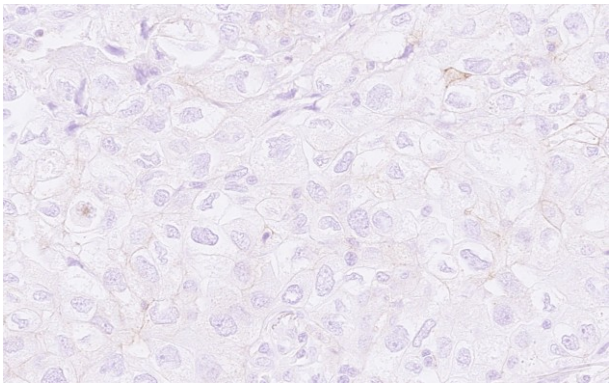


Fig. 2b
Insuffcient staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1b.
Overall, less than 50% of the tumour cells showed a membranous staining reaction and the PD-L1 category being changed from the expected TPS High to TPS Low and the tumour not being eligible for immune therapy.
Both the participating laboratory and the NordiQC assessor panel scored the result as TPS Low.
Compare to the expected result as shown in Fig. 2a.

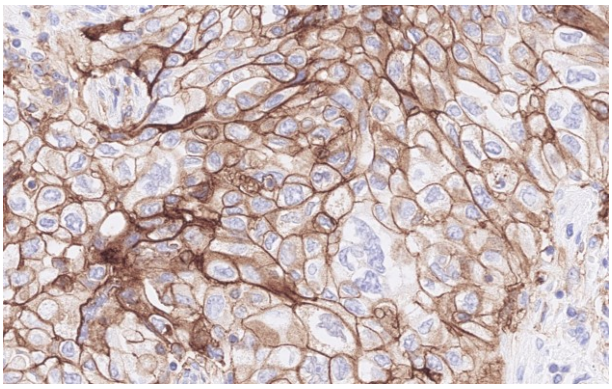


Fig. 3a
Optimal staining result of the NSCLC, tissue core no. 6, using the same protocol as in Figs. 1a and 2a.
A weak to strong, distinct membranous staining reaction is seen in virtually all tumour cells. The tumour was categorized as TPS High (≥50%) and thus eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur).

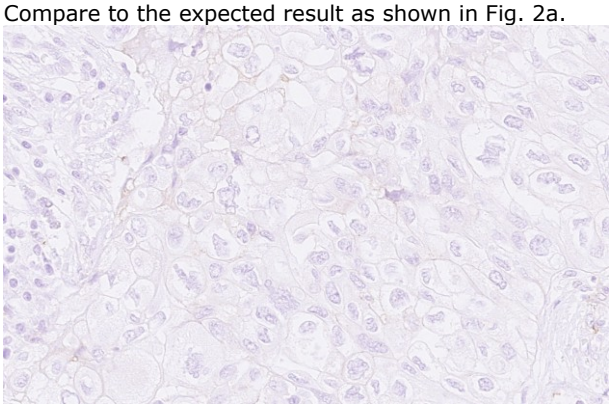


Fig. 3b
Insufficient staining result of the NSCLC, tissue core no. 6, using the same protocol as in Figs. 1b and 2b.
Less than 50% of the tumour cells show a membranous staining reaction and the PD-L1 category being changed from the expected TPS High to TPS Low and the tumour not being eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur).
Compare to the expected result as shown in Fig. 3a.

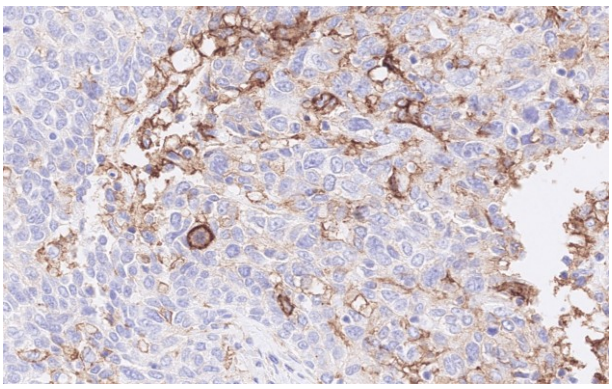


Fig. 4a
Optimal staining result of the BC, tissue core no. 9, using the same protocol as in Figs. 1a - 3a.
A weak to moderate staining reaction is seen in lymphocytes and macrophages adjacent to the tumour cells being negative.
The tumour was categorized as CPS High (≥10) and thus eligible for immune therapy with KEYTRUDA®.

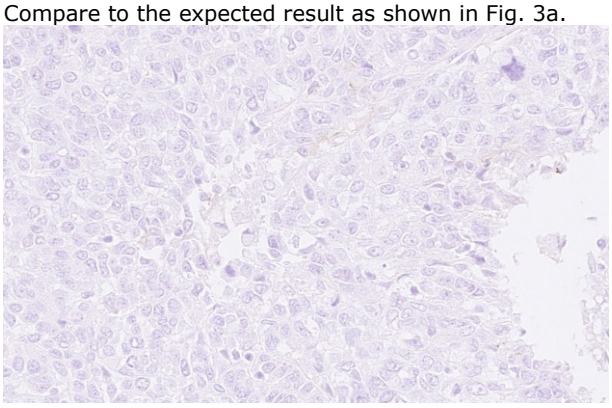


Fig. 4b
Staining result of the BC, tissue core no. 9, using the same protocol as in Figs. 1b-3b.
No staining reaction is observed and changing the PD-L1 category from the expected CPS High (≥10) to CPS Low (<10) and not eligible for immune therapy with KEYTRUDA®.
Compare to the expected result as shown in Fig. 4a.

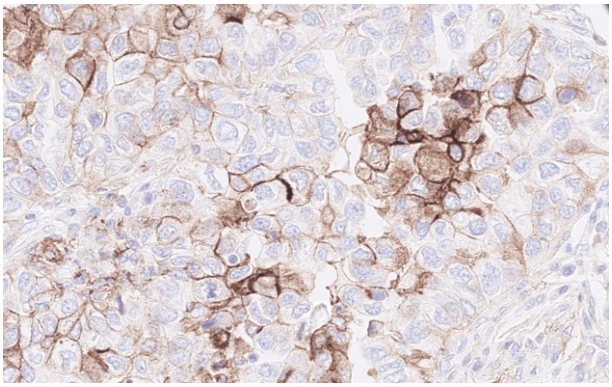


Fig. 5a
Optimal staining result of the NSCLC, tissue core no. 5, using the same protocol as in Fig. 1a.
Same tumour as in Fig. 2a, but from another TMA.
In this area a weak but distinct membranous staining reaction is seen in vast majority of tumour cells.
The result as such being categorized as TPS High (≥50%) and thus eligible for first line immune therapy with KEYTRUDA® (different regional cut-offs occur).

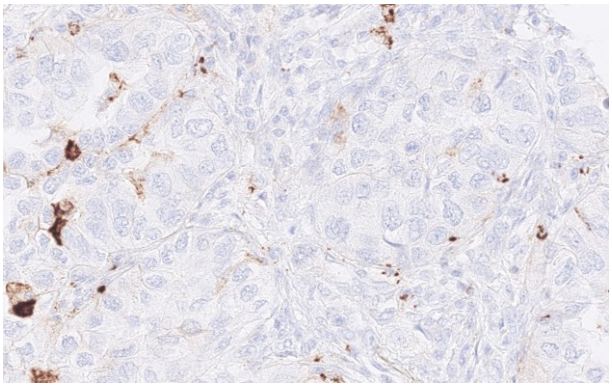


Fig. 5b
Staining result of the NSCLC, tissue core no. 5, using a modifyed protocol of the Ventana/Roche SP263 IHC assay, 741-4905, adding OptiView Amplification.
Overall, a significantly decreased proportion of tumour cells is demonstrated, changing the status from TPS High to TPS Low. In addition, the read-out is slightly compromised as the staining reaction is primarily coarsely granulated with difficulties to evaluate the PD-L1 TPS status.

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