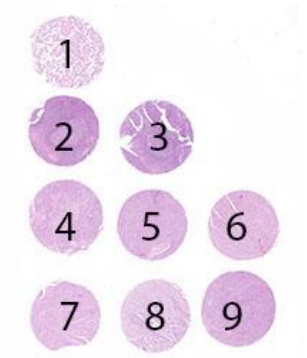
# Assessment Run C15 2024
# PD-L1 IC

**Purpose**
This assessment in the NordiQC Companion module of PD-L1 IC primarily focused on evaluation of the analytical accuracy of the PD-L1 IHC assays performed by the participating laboratories to identify patients with urothelial carcinomas or triple negative breast carcinomas (TNBC) to be treated with TECENTRIQ® as immune therapy. The PD-L1 SP142 IHC assay (741-4860, Ventana/Roche) was used as reference standard method. Accuracy was evaluated in six carcinomas with the dynamic and critical relevant expression levels of PD-L1 characterized by tumour-infiltrating immune cell score (IC). The assessment mark obtained in NordiQC is indicative of the performance of the IHC tests but due to the limited number and composition of samples, 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 IC C15 assessment**

| **Tissue controls** | **PD-L1 IHC reaction pattern** |
|---|---|
| 1. Placenta | See control section |
| 2-3. Tonsil | See control section |
| **Carcinomas** | **IC score*** |
| 4. TNBC | <1% IC |
| 5. TNBC | ≥1% (IC, 3-10%) |
| 6. TNBC | ≥1% (IC, 5-15%) |
| 7. Urothelial carcinoma | <5% IC |
| 8. Urothelial carcinoma | ≥5% (IC, 5-15%) |
| 9. Urothelial carcinoma | ≥5% (IC, 10-25%) |




* Tumour-infiltrating immune cell score (IC) determined by PD-L1 SP142 IHC (741-4860, Ventana/Roche) performed in NordiQC reference lab.

All tissues were fixed in 10% neutral buffered formalin.

**KEY POINTS FOR PD-L1 IC IMMUNOASSAYS**

- The Ventana/Roche RTU **SP142 assays** should be used with recommended protocol settings.
- Placenta and tonsil in combination are at present the best control tissues.
- **Non-SP142 assays** optimized for PD-L1 TPS/CPS status cannot be recommended for IC-scoring due to inferior performance.

The participating laboratories were asked to perform their PD-L1 IHC assay for treatment decision with TECENTRIQ®, evaluate the PD-L1 expression level using IC score as read-out method and submit the stained slides and scores to NordiQC. This allowed both an assessment of the technical performance (analytical accuracy) of the PD-L1 IHC assays but also information on the reproducibility and concordance of the PD-L1 expression read-out results among the laboratories.

**PD-L1 IC IHC, 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 made throughout the blocks. Every twenty-fifth slide was thus stained for PD-L1 using the CE IVD / FDA approved PD-L1 SP142 IHC assay (741-4860, Ventana/Roche). During the assessment, IC categories for each tissue core on the submitted slides were compared to the level in the nearest reference slide of PD-L1 (SP142).

**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>IC score 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 in all of the included tissues.
The PD-L1 expression in one or more tissues varies significantly from the expected IC scores, but still in right category.
The protocol may be optimized to ensure analytical accuracy and/or improved counter staining, morphology and signal-to-noise ratio.
<u>IC score is concordant to the NordiQC reference data in all carcinomas.</u>

**Criteria for assessing a staining as <u>Borderline</u> include:**
The staining is considered insufficient, e.g., because of a generally too weak staining, a false negative staining or a false positive staining reaction in one of the included tissues. The protocol should be optimized.
<u>IC score is **not** found 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 in more than one of the included tissues.
An optimization of the protocol is urgently needed.
<u>IC score is **not** found 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 reaction in non-immune cells hampering the read-out.

**PD-L1 IHC, Read-out**
All participating laboratories were asked to submit a scoring sheet with their read-out of the tumour-infiltrating immune cell score (IC) in the six carcinomas. Results were compared to NordiQC data from the reference laboratory to analyse scoring consensus.

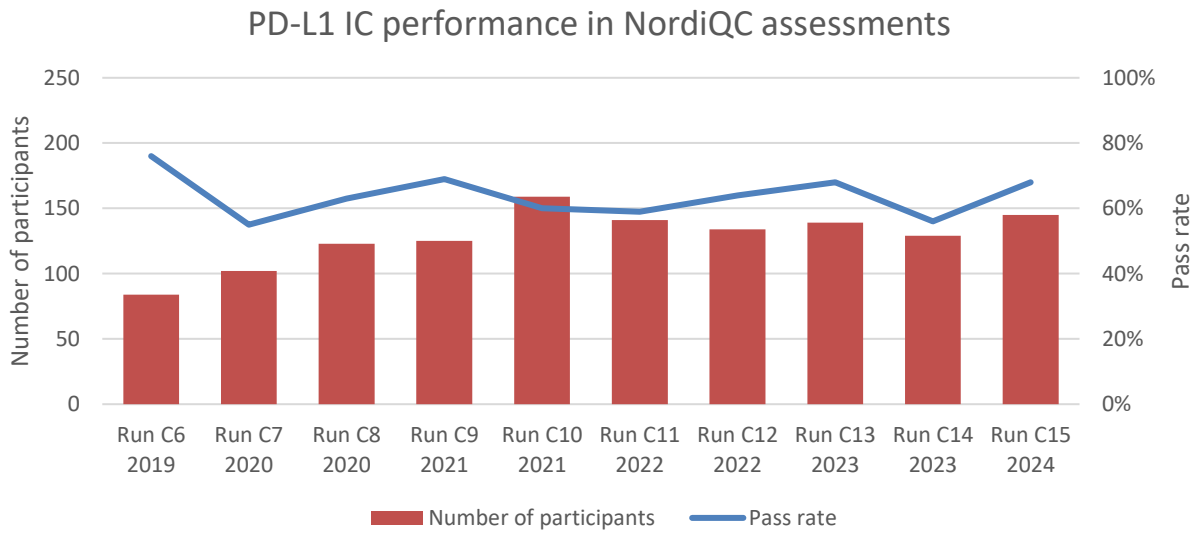
**Participation**

| | |
|---|---|
| Number of laboratories registered for PD-L1 IC IHC C15 | 162 |
| Number of laboratories returning PD-L1 IC IHC | 146 (90%) |
| Number of laboratories returning PD-L1 scoring sheet | 125 |

**Results:** 146 laboratories participated in this assessment. One laboratory submitted a wrong slide and the result was not included in the following report. Of the remaining 145, 68% achieved a sufficient mark. Assessment marks for IHC PD-L1 assays and PD-L1 antibodies are summarized in Table 2a-d (see page 3-4). All slides returned after the assessment were assessed and received advice if the result being insufficient but were not included in this report.

**Performance history**
This was the tenth NordiQC assessment of PD-L1 IC. The overall pass rate increased compared to the level seen in the last run and is comparable to the level seen in e.g. run C9, C12 and C13.

Graph 1. **Proportion of sufficient results for PD-L1 IC in the ten NordiQC runs performed.**



**Controls**
Tonsil and placenta were used as positive and negative tissue controls. In this assessment and in concordance with the official scoring guidelines from Ventana/Roche, tonsil was found to be the most appropriate and recommendable positive and negative tissue control. However, the use of tonsil as QC tool to monitor the reproducibility of the PD-L1 IC test is challenged as only a binary reaction pattern of either strongly positive or negative cells are identified and no cells in tonsil are identified with consistently low expression levels to be used as a more reliable tool to confirm IHC assay reproducibility identifiyng any test fluctuation and reduced analytical sensitivity of the PD-L1 IC test. In this context, it was observed in both this and previous assessments, that placenta might be a supplemental positive tissue control. It was as such seen that a weak to strong staining reaction in at least dispersed cytotrophoblasts in placenta, could be used to verify the appropriate and expected level of analytical sensitivity for the Ventana/Roche SP142 assays based on tyramide amplification. If these cells were identified and positive with the Ventana/Roche SP142 assays, the results in the other tissues were as expected and evaluated as successful, whereas if these cells were negative a large proportion of insufficient and false negative results in the other tissues were observed. This observation however still must be further validated.

When tonsil is used as positive and negative tissue control following pattern must be seen; The majority of crypt epithelial cells in the tonsil should display a strong staining reaction, while a moderate to strong staining reaction should be seen in many germinal center lymphocytes, macrophages and scattered immune cells in the interfollicular regions. No staining reaction should be seen in superficial squamous epithelial cells and mantle zone B-cells. As in previous assessments, it was observed that a moderate staining reaction in scattered immune cells in the interfollicular region was more challenging for the participants and could only be detected with an optimal protocol.

**Conclusion**
This was the tenth NordiQC assessment of PD-L1 for IC in urothelial carcinoma and TNBC in the companion module. 145 laboratories participated and a pass rate of 68% was observed.
The PD-L1 SP142 companion diagnostic (CDx) IHC assay product no. 741-4860 and the IHC assay 790-4860 both from Ventana/Roche were the most successful assays for the evaluation of PD-L1 status in urothelial carcinomas and TNBCs to guide treatment with TECENTRIQ® as immune therapy providing a pass rate of 92% and 95%, respectively. Other PD-L1 CDx assays as SP263 (741-4905, Ventana/Roche) and 22C3 (SK006/GE006, Dako/Agilent) being very successful in the NordiQC PD-L1 TPS/CPS assessments provided only insufficient staining results. The insufficient results were characterized by either pure false negative results (seen for SP142) or a false positive IC result of the TNBC expected to be negative and an extensive staining reaction in tumour cells in one or more of the carcinomas compromising the evaluation of PD-L1 reaction in immune cells (non-SP142 based assays).

Table 2a. **Overall results for PD-L1 IC, run C15**

| | n | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|
| CE-IVD / FDA approved PD-L1 assays | 66 | 40 | 8 | 6 | 12 | 73% | 61% |
| Antibodies for laboratory developed PD-L1 assays, based on concentrated antibodies | 10 | - | 1 | 1 | 8 | 10% | 0% |
| Ready-To-Use antibodies | 69 | 30 | 19 | 6 | 14 | 71% | 43% |
| Total | 145 | 70 | 28 | 13 | 34 | | |
| Proportion | | 48% | 19% | 9% | 23% | 68% | |

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).

Table 2b. **Assessment marks for CE-IVD / FDA approved PD-L1 assays for PD-L1 IC, run C15**

| **CE-IVD / FDA approved PD-L1 assays** | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| rmAb clone SP142, **741-4860[3]** | 51 | Ventana/Roche | 39 | 8 | 2 | 2 | 92% | 76% |
| rmAb clone SP142, **741-4860[4]** | 1 | Ventana/Roche | 1 | - | - | - | - | - |
| rmAb clone SP263, **741-4905[3]** | 6 | Ventana/Roche | - | - | 2 | 4 | 0% | 0% |
| rmAb clone SP263, **741-4905[4]** | 2 | Ventana/Roche | - | - | 1 | 1 | - | - |
| mAb clone 22C3 pharmDX, **SK006** | 2 | Dako/Agilent | - | - | - | 2 | - | - |
| mAb clone 22C3 pharmDX, **GE006** | 4 | Dako/Agilent | - | - | 1 | 3 | - | - |
| Total | 66 | | 40 | 8 | 6 | 12 | | |
| Proportion | | | 61% | 12% | 9% | 18% | 73% | |

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) This product has a locked protocol on all BenchMark platforms and cannot be changed.
4) RTU product applied on another platform than developed for.

Table 2c. **Assessment marks for concentrated antibodies for PD-L1 IC, run C15**

| **Antibodies[7] for laboratory developed PD-L1 assays, concentrated antibodies** | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **22C3** | 5 | Dako/Agilent | - | 1 | 1 | 3 | 20% | 0% |
| rmAb clone **CAL10** | 1<br>1 | Zytomed<br>Biocare Medical | - | - | - | 2 | - | - |
| rmAb clone **E1L3N** | 3 | Cell Signaling | - | - | - | 3 | - | - |
| Total | 10 | | - | 1 | 1 | 8 | | |
| Proportion | | | 0% | 10% | 10% | 80% | 10% | |

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

Table 2d. **Assessment marks for Ready-To-Use antibodies[8] for PD-L1 IC, run C15**

| **Ready-To-Use antibodies[8]** | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| rmAb clone SP142, **790-4860 (VRPS)[5]** | 22 | Ventana/Roche | 17 | 4 | 1 | - | 95% | 77% |
| rmAb clone SP142, **790-4860 (LMPS)[6]** | 33 | Ventana/Roche | 13 | 15 | 3 | 2 | 85% | 39% |
| rmAb clone SP263, **790-4905/740-4907** | 10 | Ventana/Roche | - | - | 1 | 9 | 0% | 0% |
| rmAb clone SP142, **RMA-0724** | 1 | Fuzhou Maixin | - | - | - | 1 | - | - |
| rmAb clone MSVA-711R, **MSVA-711R** | 1 | MS Validated Antibodies | - | - | 1 | - | - | - |
| rmAb clone GR110, **GT256202** | 1 | Gene Tech | - | - | - | 1 | - | - |
| rmAb clone E1L3N **P06B01** | 1 | MEDx Translational Medicine | - | - | - | 1 | - | - |
| Total | 69 | | 30 | 19 | 6 | 14 | | |
| Proportion | | | 43% | 28% | 9% | 20% | 71% | |

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
5) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.
6) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.
8) Ready-To-Use antibodies without predictive claim.

**Detailed Analysis**
**CE IVD / FDA approved assays**
**SP142** (741-4860, Ventana/Roche): In total, 39 of 51 (76%) 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) for 48 min., 16 min. incubation of primary Ab and OptiView with OptiView Amplification as detection system. Using these protocols settings and applied on a BenchMark platform, 47 of 51 (92%) laboratories produced a sufficient staining result (optimal or good).

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used CDx assays with a predictive claim. The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the specific IHC stainer device are included.

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

| CDx assays | **Vendor recommended protocol settings[1]** | | **Laboratory modified protocol settings[2]** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Ventana BenchMark GX, XT, Ultra rmAb SP142, **741-4860** | 47/51 (92%) | 39/51 (76%) | - | - |
| Ventana BenchMark GX, XT, Ultra rmAb SP263, **741-4905** | 0/6 (0%) | 0/6 (0%) | - | - |
| Dako Autostainer Link 48+ mAb 22C3 pharmDX, **SK006** | 0/2 | 0/2 | - | - |
| Dako Omnis mAb 22C3 pharmDX, **GE006** | 0/4 | 0/4 | - | - |

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

**Ready-To-Use antibodies for laboratory developed (LD) assays**
**SP142** (790-4860, Ventana/Roche): In total, 30 of 55 (55%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 24-64 min.), 16-32 min. incubation of primary Ab and OptiView with OptiView Amplification as detection system. Using these settings, 46 of 48 (96%) produced a sufficient staining result.

**Block construction and assessment reference standards**
The tissue micro array (TMA) blocks constructed for this PD-L1 IC run consisted of three urothelial carcinomas, three TNBCs, two tonsils and one placenta. The three urothelial carcinomas were selected to comprise one carcinoma with an IC score <5% and two with IC score ≥5%. The three TNBCs were selected to comprise one carcinoma with an IC score <1% and two with IC score ≥1%. For the two entities the positive IC score characterized by both aggregate and single cell staining pattern.
Reference slides throughout the individual TMA blocks (interval at each twenty-fifth slide) were stained using the companion diagnostic assay SP142, (741-4860, Ventana/Roche).
In total, five identical TMA blocks were constructed and used for this assessment. The quality of the sections circulated, was in this assessment less sufficient, as many folds were seen due to paraffin/agar issues in the recipient TMA blocks. The folds had no impact on the assessment of PD-L1 results in the slides.
During the assessment, IC scores 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 and the assessment in this sense emulated clinical settings.

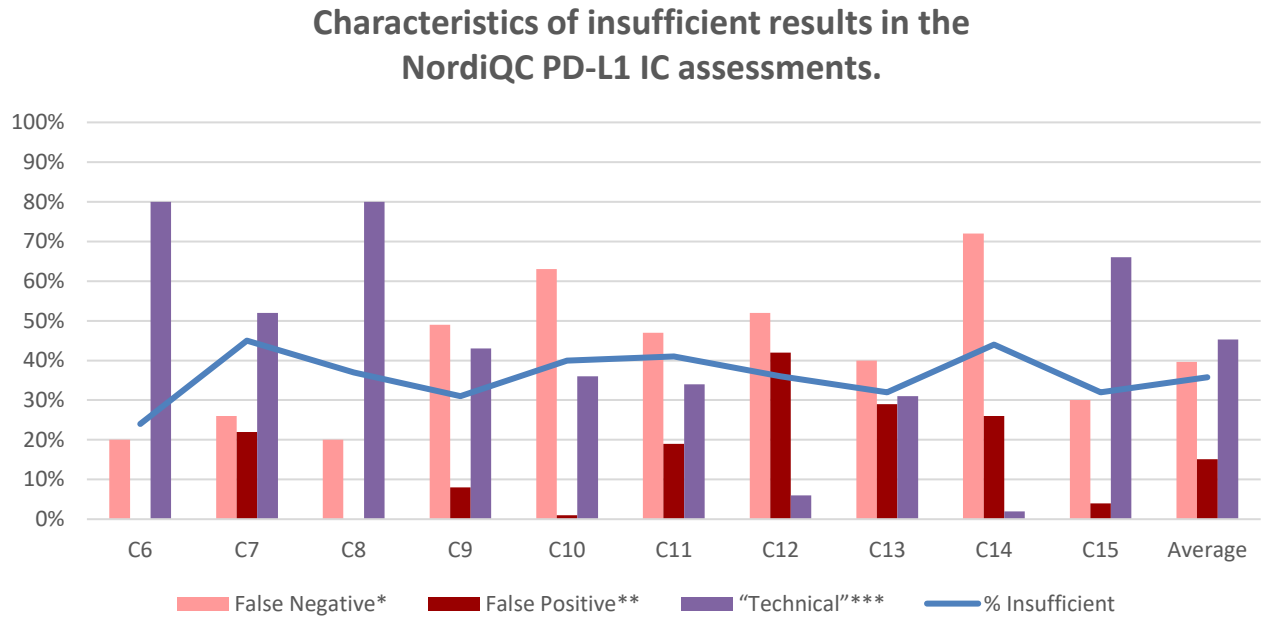
**Comments – accuracy of PD-L1 IHC using IC scoring to guide treatment with TECENTRIQ®**

In this tenth NordiQC run C15 for PD-L1 IC in the companion module, a pass rate of 68% was observed for the participants performing PD-L1 IHC assays to identify patients with urothelial carcinomas and TNBCs to be treated with TECENTRIQ® as immune therapy using the IC scoring method.
The pass rate, as shown in Graph 1 (see page 2), significantly increased compared to the level seen in the previous runs C14 and is similar to the level seen in run C9. The central parameters potentially affecting pass rates in IHC proficiency schemes were identical in all runs. Of critical importance, the same assessment criteria, reference standard methods and scoring guidelines were applied. The materials / carcinomas selected were different in the individual runs, but all selected to represent the same diagnostic relevant cut-off levels being verified by the approved SP142 diagnostic assay. In addition, all included

tissue material have been processed accordingly to the standard operating procedures described for PD-L1 IHC testing.

It was observed that insufficient results were most frequently characterized by a reduced proportion of cells demonstrated or a completely false negative staining reaction of immune cells in one or more of the tissue cores and was seen in 30% (14 of 47) of the insufficient results. This was especially observed in the TNBC tissue core no. 5 and the urothelial carcinoma tissue core no. 8 and confirmed by both the read-out performed by the NordiQC assessors and the participants submitting scoring sheets – see Graph 4, page 8. In 4% (2 of 47) the insufficient staining result was caused by an increased proportion of immune cells in one of the PD-L1 negative tumours giving a false positive staining reaction. In 57% (27 of 47) of the insufficient results, an excessive staining reaction of tumour cells compromising the scoring and PD-L1 status in the immune cells was seen. The excessive staining reaction observed in tumour cells, complicating the read-out was also seen in the two false positive results. In the remaining 9% (4 of 47) of the insufficient results, either poor signal-to-noise ratio or excessive background were seen complicating the read-out. Graph 2 shows the main characteristics of insufficient results in the ten NordiQC PD-L1 IC runs performed.

Graph 2. **Prevalence and characteristics of insufficient results.**




* IC score change from positive to negative in one or more of the included carcinomas.
** IC score change from negative to positive in one or more of the included carcinomas.
*** Read-out compromised e.g. by poor-signal-to noise ratio, poor morphology, excessive cytoplasmic staining reaction etc.

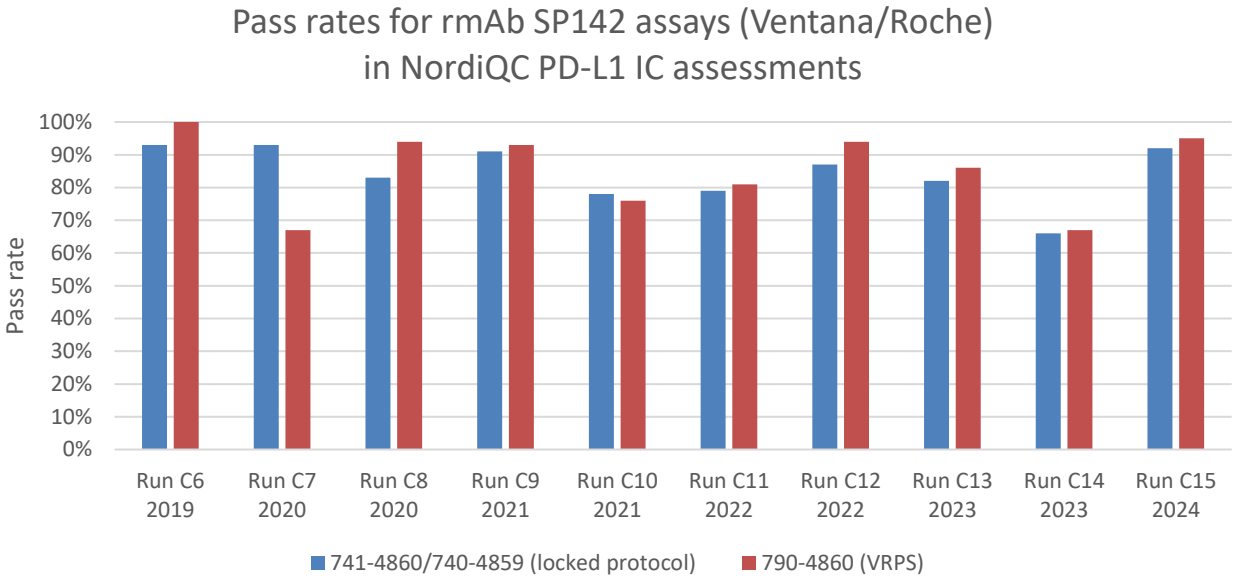
The Ventana/Roche PD-L1 SP142 assay 741-4860 with predictive claim for TECENTRIQ® was used by 35% of the participants and provided a pass rate of 92%. The assay is locked for central protocol settings and based on HIER in CC1 for 48 min., incubation in primary Ab for 16 min. (Ultra/XT/GX) and use of OptiView with Amplification as detection system. Despite the locked protocol conditions for the assay, some laboratories submitted protocols with reported modified settings indicating change in efficient heating time of HIER, primary Ab and other detection system applied – e.g. OptiView without Amplification. The various protocol settings submitted were disregarded for the assay product no. 741-4860 in this report and all protocols thus compiled as used by vendor recommended protocol settings as shown in Tables 2 and 3.

The Ventana/Roche PD-L1 SP142 assay 790-4860 without any predictive claim and available as an analytical or generic PD-L1 assay was used by 34% of the participants on the intended platform. This assay is based on same recommended protocol settings as the corresponding CDx product 741-4860, but with ordinary options for the laboratories to modify the protocol settings in their optimization and validation process for the implementation of the test. Overall, the SP142 790-4860 format gave a pass rate of 96% being similar to the locked assay. A similar pass rate and proportion of optimal results was obtained, both when using the vendor recommended- and modified protocol settings, compared to the CDx format 741-4860 of the same clone as seen in Table 2b + 2d (see page 4-5).

In this run, the two Ventana/Roche PD-L1 SP142 assays 741-4860 and 790-4860 have provided a significantly increased pass rate compared to the latest runs and are now on the level seen in runs C9 and

C12. As seen in Graph 3, fluctuations in pass rates for the Ventana/Roche SP142 has been seen in all 10 runs. No plausible reason for this has been identified. As the IHC assay and PD-L1 result is based on multiple elements primary focus should be on the most critical parameters as different lots of primary antibody, detection systems incl. the amplification kits and/or bulk reagents. In addition to the focus on the analytical parameters causing the low reproducibility, other aspects as poor tissue quality/handling, inappropriate sectioning of slides circulated, quality of the coated slides used by NordiQC should be addressed. In this context NordiQC performed 23 PD-L1 reference slides throughout the TMAs used for this assessment run and all passed internal evaluation as sufficient.

Graph 3. **Pass rates for the rmAb SP142 assays (Ventana/Roche) in the NordiQC assessments.**



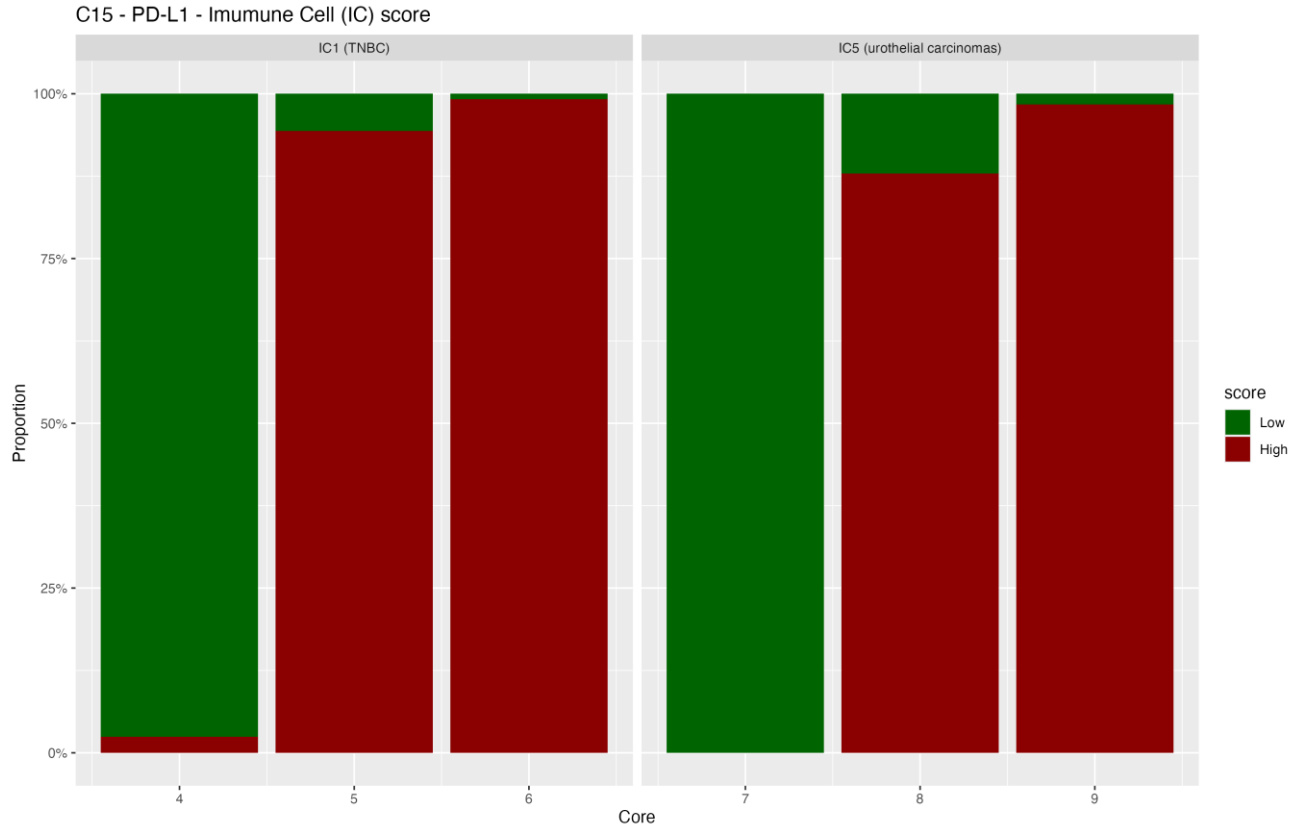

Laboratories obtaining an insufficient score are recommended to continue to use the two SP142 based PD-L1 assays with vendor recommended protocol settings, as they historically in the NordiQC assessments have generated high qualitative results, but also highly encouraged to perform in-house metrics of the PD-L1 results obtained to monitor and document these and hereby verify the proportion of positive and negative results being on par to levels expected and published for the cancer types in question.
At this point it also has to be underlined that despite tonsil is the recommended and at present most reliable positive and negative tissue control with expected test performance characteristics and reaction pattern for quality control (QC) of PD-L1 IC testing, this might be challenging in real life QC. The challenges primarily related to a binary strongly positive or negative staining reaction of immune cells and epithelial cells in the tonsil, with no cells identified with low expression levels to be used as critical controls to monitor the low limit of PD-L1 demonstration. Without such tool, the ability to evaluate the analytical precision and reproducibility of the PD-L1 IHC test is hampered and e.g. difficult to identify if a fluctuation of the IHC test system for PD-L1 occurs.
In same context, it has to be emphasized that external and central parameters potentially affecting pass rates in IHC proficiency schemes have been identical in all the ten NordiQC assessment runs for PD-L1 IC. Of critical importance, the same assessment criteria, reference standard methods and scoring guidelines were applied. The materials / carcinomas selected and used for the individual assessment runs are different and variations in pass rates might be caused by more or less challenging material circulated in the individual runs. However, in this context, it has to be mentioned that the included materials all have been processed concordantly to guidelines for PD-L1 IHC testing, and the expression levels being verified throughout all the TMA's used for the assessments.

"Non-SP142" companion diagnostic assays as SP263 (Ventana/Roche) and 22C3 pharmDx (Dako/Agilent), but also laboratory developed (LD) assays based on either concentrated primary Abs or RTU formats gave an overall significantly inferior performance and reduced pass rate at 3% (1 of 38), none optimal, compared to the SP142 assays from Ventana/Roche used on the Ventana BenchMark platforms.
The majority (71%) of the insufficient results for "non-SP142" assays provided an extensive staining reaction of tumour cells compromising the scoring of PD-L1 expression in immune cells.
Similar observations were seen in runs C6-C14, and these data indicate a challenge for the interchangeability of the Ventana SP142 assays with other PD-L1 companion diagnostic assays and LD assays most likely designed and developed to primarily provide a staining pattern as characterized by e.g. the Dako/Agilent 22C3 pharmDx assays. One of the most influencing causes for the inferior performance

of "non-SP142" assays seem to be related to the detection system applied for the Ventana SP142 assays being based on OptiView with Amplification kit (tyramide based) and the calibration of the SP142 antibody in the Ventana/Roche assays provides a performance that intensifies demonstration of immune cells and reduces staining of tumour cells.
This consideration and conclusion is fully in line with the publication of Kelly A. Schatts et al (Optimal Evaluation of Programmed Death Ligand-1 on Tumour Cells Versus Immune Cells Requires Different Detection Methods, Arch Pathol Lab Med. 2018 Aug;142(8):982-991) stressing that *"diverse sensitivities caused by the choice of the detection method should be taken into consideration when selecting PD-L1 kits or developing PD-L1 IHC laboratory-developed tests.".* Only by using the same detection system OptiView with Amplification, the classical clones as 22C3 and 28-8 could provide staining patterns largely comparable to the Ventana/Roche SP142 assays. In general, a PD-L1 IHC test must be fit-for-purpose aligning treatment, indication, scoring system and PD-L1 IHC assay.
When using alternative companion diagnostic assays or LD assays, it is strongly recommended to compare and validate these with the original assay.
The meta-analysis for PD-L1 accuracy by Torlakovic et al; "Interchangeability" of PD-L1 immunohistochemistry assays: a meta-analysis of diagnostic accuracy. Modern Pathology (2020) 33:4–17 also indicates that in-house or laboratory developed PD-L1 IHC assays must be developed and validated against the reference standard and approved companion diagnostic assay.

In this NordiQC PD-L1 IHC segment for IC score, the SP142 CDx assay is used as reference standard method using the associated approved read-out criteria. The results of the participants are compared directly one-to-one to the reference levels. The assessment marks only address the analytical concordance using the approved cut-off and read-out criteria focusing on IC score and e.g. application of alternative scoring systems and cut-off's for non-SP142 CDx assays are not included to adjust any option for interchangeability.

**PD-L1 scoring**
Participants were asked to evaluate the IC score in each of the three TNBC (IC with 1% cut-off) and three urothelial carcinomas (IC with 5% cut-off) included in the assessment material. The overall read-out of the PD-L1 expression among the participants is shown in Graph 4.




Graph 4. **NordiQC PD-L1 run C15: Read-out of IC in three TNBC and three urothelial carcinomas.**

As seen in Graph 4, a relatively high consensus rates were observed in tissue cores no 4, 6, 7 and 9. The reduced consensus rate in tissue cores no 5 and 8 reflects the observation that these two tissues cores in

the vast majority of insufficient results were characterized with a PD-L1 IC score <1% and <5%, respectively.

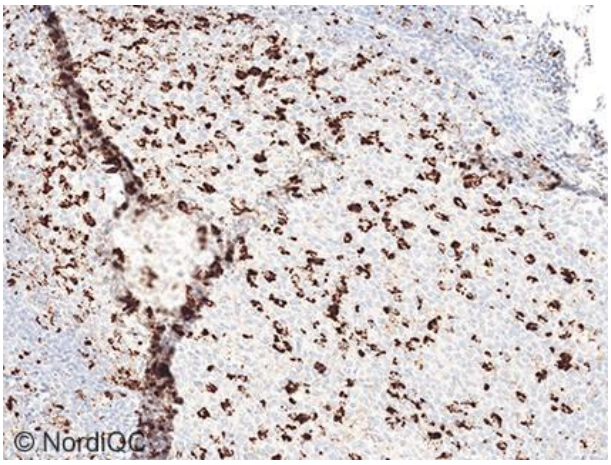



Fig. 1a
Optimal staining result of tonsil using the PD-L1 IHC assay 741-4860 from Ventana/Roche, based on the rmAb clone SP142 following the recommended protocol settings. Same protocol used in Figs. 2a-6a.
Many germinal centre lymphocytes/macrophages and scattered interfollicular immune cells show a moderate to strong staining reaction. As mentioned, the quality of circulated TMA sections was hampered by folds, but still the PD-L1 IHC results could be scored with confidence.

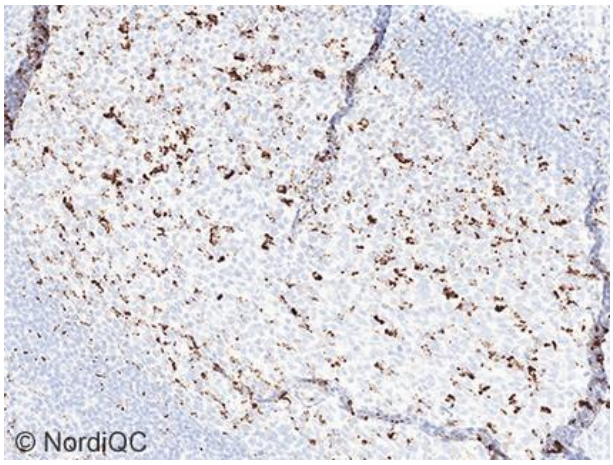



Fig. 1b
Staining result of tonsil using the PD-L1 IHC assay 741-4860 from Ventana/Roche, based on the rmAb clone SP142 following the recommended protocol settings and same as applied in Figs. 1a-6a. Overall a reduced analytical and diagnostic sensitivity was observed, but no identification of root cause for this aberrant result.
Same protocol used in Figs. 2b-4b.
The staining intensity and proportion of immune cells is reduced compared to the optimal result in Fig. 1a.

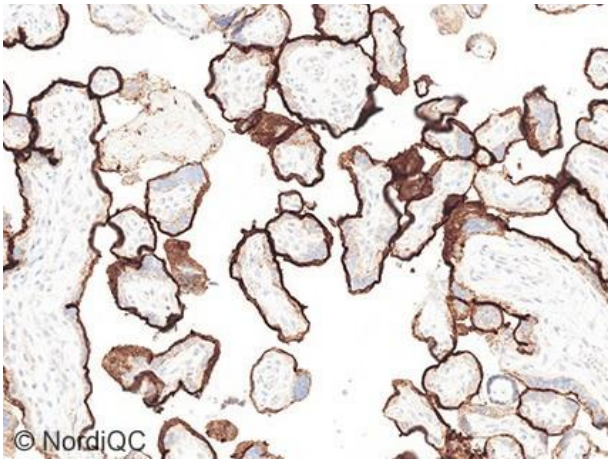



Fig. 2a
Optimal staining result of placenta using the same protocol as in Fig. 1a and providing the expected results in all the included tissues/neoplasias.
Most trophoblasts show a weak to strong membranous staining reaction.

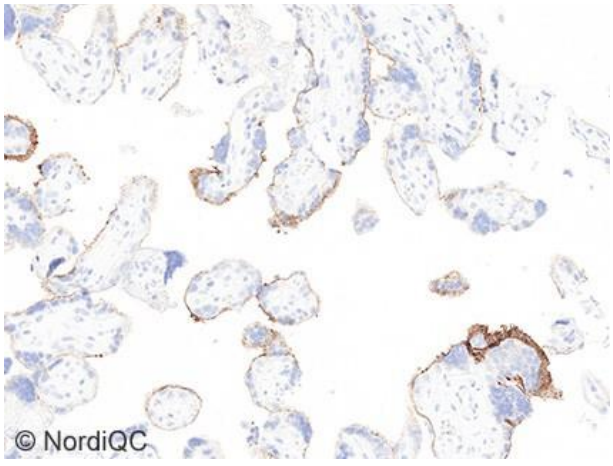



Fig. 2b
Insufficient staining result of placenta using same protocol as in Fig. 1b giving an insufficient result in many of the included neoplasias.
The trophoblasts are virtually negative.
Compare with Fig. 2a – same area.

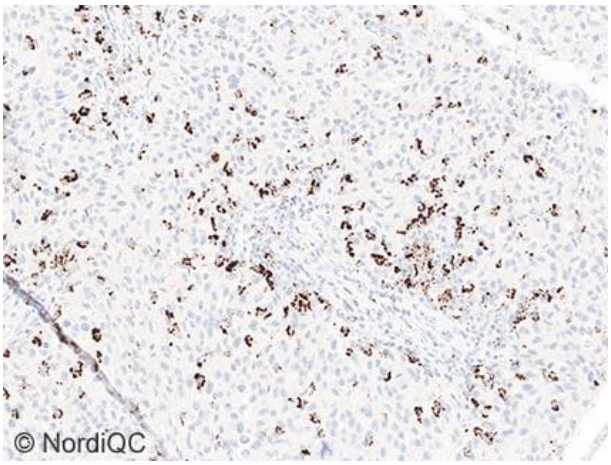



Fig. 3a
Optimal staining result of the TNBC, tissue core no. 5, using same protocol as in Figs. 1a and 2a. Virtually all tumour cells are negative and immune cells show a moderate to strong staining reaction giving an IC score of ≥1%.

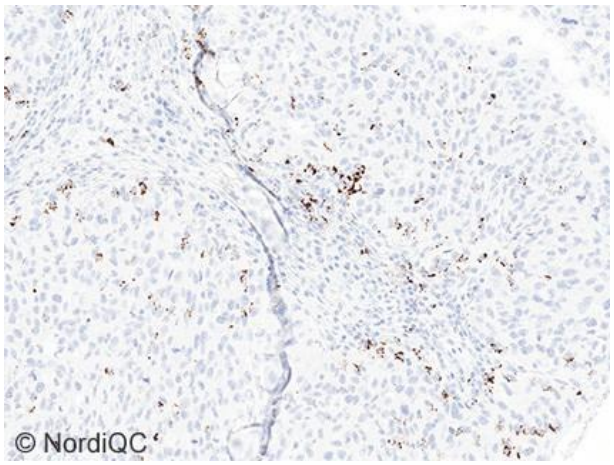



Fig. 3b
Staining result of the TNBC, tissue core no. 5, using same protocol as in Figs. 1b and 2b. The proportion of positive cells is significantly reduced. Also compare the result in Fig. 4b, same protocol.

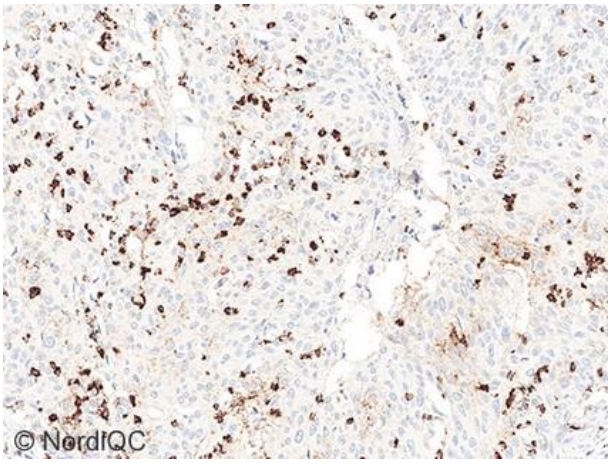



Fig. 4a
Optimal staining result of the urothelial carcinoma, tissue core no. 8, using same protocol as in Figs. 1a-3a. Immune cells display a moderate to strong staining reaction giving an IC score ≥5%.

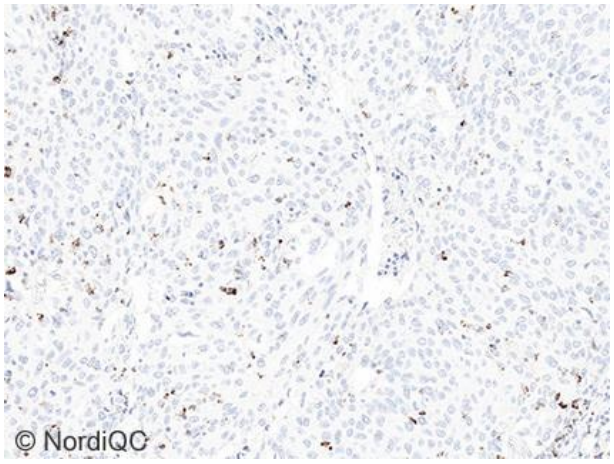



Fig. 4b
Insufficient staining result of the urothelial carcinoma, tissue core no. 8, using same protocol as in Figs. 1b-3b. An IC score of <5% is obtained changing the PD-L1 category from positive to negative. Compare to the optimal result shown in Fig. 4a – same area.

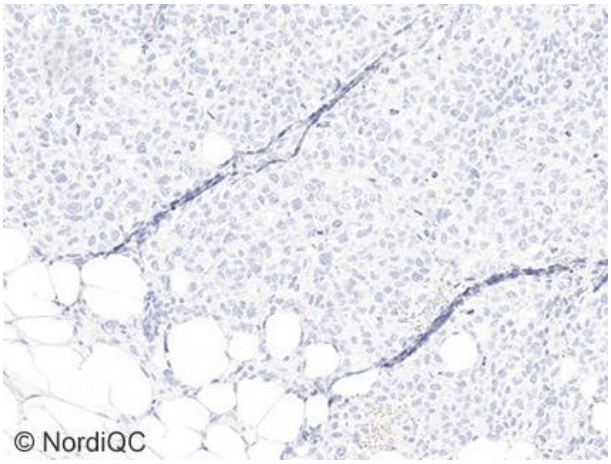



Fig. 5a
Optimal staining result of the TNBC, tissue core no. 4, using same protocol as in Figs. 1a–4a. Virtually all tumour cells and immune cells are negative giving an IC score of <1%.

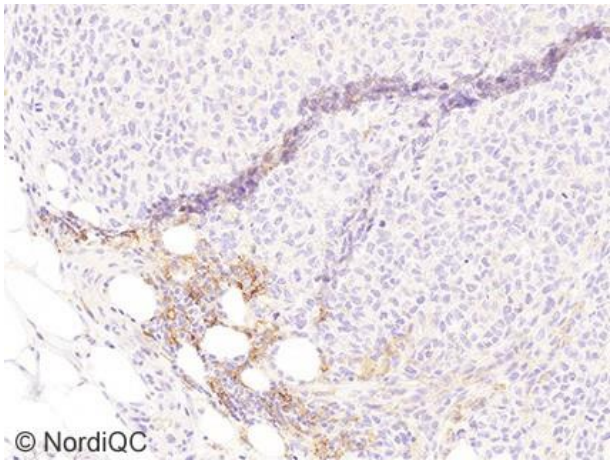



Fig. 5b
Insufficient staining result of the TNBC, tissue core no. 4, using the rmAb SP263.
An IC score of ≥1% is obtained changing the PD-L1 category from negative to positive. Compare to the optimal result shown in Fig. 5a – same area. The protocol is most likely calibrated to identify PD-L1 in tumour cells e.g. for TPS in NSCLC.

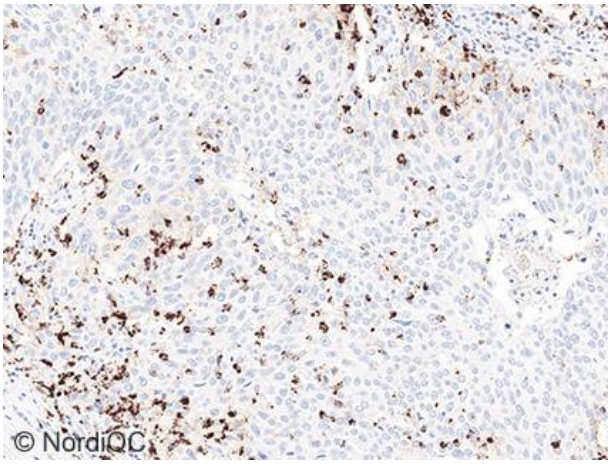



Fig. 6a
Optimal staining result of the urothelial carcinoma, tissue core no. 8, using same protocol as in Figs. 1a-5a. Immune cells display a moderate to strong staining reaction giving an IC score ≥5% (The absence of staining reaction in the tumour cells facilitates the evaluation of PD-L1 IC score.

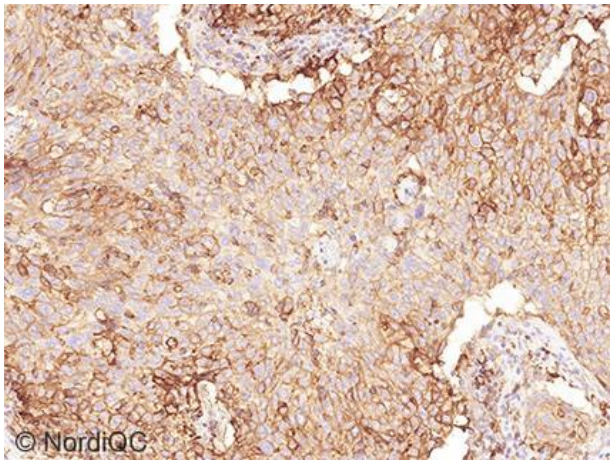



Fig. 6b
Insufficient staining result of the urothelial carcinoma, tissue core no. 8, using same protocol as in Fig. 5b.
The majority of tumour cells display a weak to moderate, granular membranous staining reaction compromising the identification and evaluation of PD-L1 reaction in the immune cells. Compare to the optimal result shown in Fig. 6a – same area.

HLK/LE/SN 26.06 2024