Assessment Run C5 2019

PD-L1 (uro)

This first assessment in the NordiQC PD-L1 (uro) Companion module primarily focused on the accuracy of the PD-L1 (uro) IHC assays performed by the participating laboratories to identify patients with urothelial carcinomas to be treated with KEYTRUDA® as immune therapy. The selection of urothelial carcinomas was performed to represent different clinical relevant levels of PD-L1 expression characterized and evaluated by a combined positive score (CPS). The PD-L1 expression levels in the circulated material used for the assessment were primarily characterized by the CE IVD approved companion diagnostic IHC assay, 22C3 pharmDx, SK006 Dako/Agilent for KEYTRUDA® in urothelial cancers, but also evaluated by other CE IVD assays 28-8 pharmDx, SK005 Dako/Agilent and Ventana PD-L1 (SP263) assay, 790-4905 despite not being approved for KEYTRUDA® in urothelial cancers (present status in EU). The PD-L1 evaluation in the individual tissue cores and levels were based on the cut-off values accordingly to the interpretation guideline for the 22C3 pharmDx assay SK006 for urothelial carcinoma, indicating a CPS of ≥10 as cut-off level for being "positive" and CPS <10 being "negative".
The module was designed for urothelial cancers to be characterized by CPS for KEYTRUDA® and not designed for immune cell (IC) score to guide treatment with TECENTRIQ®. However, subsequently to the design and construction of the material for the PD-L1 (uro) assessment, NordiQC realized that a relatively high proportion of laboratories used the CE IVD approved assay SP142, Ventana with the associated IC score for urothelial cancers and treatment with TECENTRIQ®. Subsequently, NordiQC initiated a process to characterize the PD-L1 expression and IC score status using the companion diagnostic assay 741-4860 SP142, Ventana in the TMA material used in the assessment. Challenges to generate reproducible and reliable expression levels in the NordiQC reference laboratory prevented the assessment of 14 laboratories using SP142, Ventana for PD-L1 and IC status.

Material

Table 1. **Content of the multi-block used for the NordiQC PD-L1 (uro) C5 assessment**

	PD-L1 IHC reaction pattern
Tissue controls	
1. Placenta	See section for controls
2-3. Tonsil	See section for controls
Urothelial carcinomas	**CPS score* & cell types being positive**
4. Urothelial carcinoma	≥10 (Immune cells)
5. Urothelial carcinoma	<10
6. Urothelial carcinoma	<10
7. Urothelial carcinoma	≥10 (Immune cells + tumour cells)
8. Urothelial carcinoma	≥10 (Tumour cells)
9. Urothelial carcinoma	≥10 (Tumour cells)
10. Urothelial carcinoma	≥10 (Immune cells + tumour cells)

* Combined positivity score (CPS) determined by PD-L1 IHC 22C3, SK006 Dako performed in NordiQC reference lab.

All tissues were fixed in 10% neutral buffered formalin.

The participating laboratories were asked to perform the PD-L1 IHC assay accordingly to the protocol being used in the laboratory and also to interpret the PD-L1 expression level using CPS as scoring method and submit these 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 interpretation of PD-L1 expression among the laboratories.

PD-L1 (uro) IHC, Technical assessment
In order to account for heterogeneity of PD-L1 expression in the individual tumour cores included in the tissue TMA blocks, reference slides were made through out the blocks. First, middle and last slides were stained for PD-L1 using the CE IVD / FDA approved 22C3 pharmDx kit SK006 (Dako). During the assessment, CPS categories for each tissue core on the submitted slides were compared to the level in the nearest reference slide of 22C3 pharmDX SK006 (Dako). In addition, a laboratory developed IHC assay based on mAb clone 22C3 was used on every twentieth slide sent to the participants securing the quality and verifying the PD-L1 status in the circulated materiel.

Criteria for assessing a staining as Optimal include:
The staining is considered perfect or close to perfect in all of the included tissues.
CPS is concordant to the NordiQC reference data is obtained in all 7 urothelial carcinomas.

Criteria for assessing a staining as Good include:
The staining is considered acceptable in all of the included tissues. However, the protocol may be optimized to provide improved analytical accuracy, counter staining, morphology or signal-to-noise ratio.
CPS is concordant to the NordiQC reference data is still obtained in all 7 urothelial carcinomas.

Criteria for assessing a staining as Borderline 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 of one of the included tissues. The protocol should be optimized.
CPS is **not** found concordant to the NordiQC reference data in all 7 urothelial carcinomas.

Criteria for assessing a staining as Poor include:
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.
CPS is **not** found concordant to the NordiQC reference data in all 7 urothelial carcinomas.

PD-L1 IHC, Interpretation
All participating laboratories were asked to submit a scoring sheet with their interpretation of the combined positivity score (CPS) in the seven urothelial carcinomas. Results were compared to NordiQC data from the reference laboratory to analyse scoring consensus.

Participation

Number of laboratories registered for PD-L1 (uro) IHC C5	114
Number of laboratories returning PD-L1 (uro) IHC	107 (94%)*
Number of laboratories returning PD-L1 (uro) scoring sheet	94 (88%)*

*14 laboratories used the assay SP142, Ventana. Those laboratories are not included in the following analysis of this PD-L1 (uro) NordiQC assessment.

Results: 93 laboratories participated in this assessment and 76% achieved a sufficient mark. Assessment marks for IHC PD-L1 assays and PD-L1 antibodies are summarized in Table 2.

Conclusion
This was the first NordiQC assessment of PD-L1 for urothelial cancer in the companion module. 93 laboratories participated and a pass rate of 76% was observed.
For PD-L1 status in urothelial carcinomas to guide treatment with KEYTRUDA® as immune therapy, the PD-L1 22C3 IHC assay, SK006 was most successful giving a pass rate of 91%. Grouped together, the PD-L1 IHC assays (22C3 pharmDx SK006, GE006 Dako/Agilent, 28-8 pharmDx, SK005 Dako/Agilent, Ventana PD-L1 (SP263) assay, 790-4905) gave a pass rate of 90% using vendor recommended protocol settings. It has to be mentioned that only 22C3 SK006 is approved as a companion diagnostic assay for KEYTRUDA® in urothelial cancers (present status in EU).
LD assays provided a lower pass rate of 70%. Insufficient results were typically characterized by a reduced CPS compared to the level expected.
Tonsil is at present the preferred choice as positive and negative tissue control for IHC methods with the purpose to demonstrate PD-L1 for KEYTRUDA® using CPS as scoring method. The majority of epithelial crypt cells must show a moderate to strong staining reaction, while the germinal centre macrophages a weak to moderate membranous staining reaction. No staining must be seen in the vast majority of lymphocytes.

Table 2. **Assessment marks for IHC assays and antibodies run C5, PD-L1 URO IHC for CPS scoring**

CE-IVD / FDA approved PD-L1 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb clone SP263, **740-4907**	4	Ventana/Roche	3	1	-	-	-	-
rmAb clone SP263, **741-4905**	1	Ventana/Roche	-	1	-	-	-	-
rmAb clone SP263, **790-4905**	19	Ventana/Roche	11	5	2	1	84%	94%
mAb clone 22C3 pharmDX, **SK006**	11	Dako/Agilent	8	2	-	1	91%	91%
mAb clone 22C3 pharmDX, **SK006**[3]	8	Dako/Agilent	5	2	1	-	88%	-
mAb clone 22C3 pharmDX, **GE006**	3	Dako/Agilent	2	-	1	-	-	-
mAb clone 22C3 pharmDX, **GE006**[3]	1	Dako/Agilent	-	1	-	-	-	-
rmAb clone 28-8 pharmDX, **SK005**	3	Dako/Agilent	2	-	1	-	-	-
Antibodies[4] for laboratory developed PD-L1 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **22C3**	21	Dako/Agilent	7	7	3	4	67%	74%
mAb clone **E1L3N**	4	Cell Signaling	-	1	2	1	-	-
rmAb **CAL10**	3 1	Biocare Zytomed Systems	2	1	-	1	-	-
rmAb clone **28-8**	3	Abcam	1	1	-	1	-	-
rmAb clone **ZR3**	1 1 1 1	Cell Marque Zeta Corporation Nordic Biosite Gene Tech	2	-	1	1	-	-
rmAb clone **QR1**	1 1	Quartett Diagomics	1	-	-	1	-	-
rmAb **BSR90**	1	Nordic Biosite	1	-	-	-	-	-
rmAb clone **SP142**	1	Spring Biosystems	1	-	-	-	-	-
Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
rmAb CAL10, **API3171**	1	Biocare	1	-	-	-	-	-
rmAb 73-10, **PA0832**	1	Leica Biosystems	-	1	-	-	-	-
rmAb clone MXR003**, RMA-0732**	1	Maixin	-	1	-	-	-	-
Total	93		47	24	11	11		
Proportion			50%	26%	12%	12%	76%	

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 Agilent/Dako's semi-automated systems (Autostainer Link48) but used by laboratories on different platforms.
4) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody.

Detailed Analysis

CE IVD / FDA approved assays

PD-L1 IHC 22C3 pharmDx (SK006, Dako): 8 of 11 (73%) protocols were assessed as optimal. Protocols with optimal results were based on heat induced epitope retrieval (HIER) in EnVision™ Flex target retrieval solution low pH 6.1 (SK006) at 95-99°C for 20 min. in PT Link and 30 min. incubation of the primary Ab, linker and polymer and performed on the Autostainer Link 48. Using these protocol settings, 10 of 11 (91%) laboratories produced a sufficient staining result (optimal or good).

SP263 (790-4905/740-4907, Ventana): 14 of 23 (61%) protocols were assessed as optimal. Protocols with optimal results were typically based on HIER in Cell Conditioning 1 (CC1) (efficient heating time 52-64 min.), 16-24 min. incubation of the primary Ab and OptiView as detection kit and performed on the BenchMark XT/Ultra. Using these protocol settings, 20 of 21 (95%) laboratories produced a sufficient staining result.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. 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]**	
KEYTRUDA®	Sufficient	Optimal	Sufficient	Optimal
Dako Autostainer Link 48+ mAb 22C3 pharmDX, **SK006**	10/11 (91%)	8/11 (73%)	-	-
Dako Omnis mAb 22C3 pharmDX, **GE006**	1/1	1/1	1/2	1/2
Dako Autostainer Link 48+ mAb 28-8 pharmDX, **SK005[3]**	1/2	1/2	1/1	1/1
Ventana BenchMark Ultra rmAb SP263, **740-4907[4]**	3/3	2/3	1/1	1/1
Ventana BenchMark XT, GX, Ultra rmAb SP263, **790-4905**[5]	10/11 (91%)	7/11 (64%)	6/8 (75%)	4/8 (50%)
Ventana BenchMark XT, GX, Ultra rmAb SP263, **741-4905[4]**	1/1	0/1	-	-

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 above mentioned parameters. Only protocols performed on the specified vendor IHC stainer are included.
3) Protocol settings recommended by Dako for non-squamous NSCLCs for OPDIVO.
4) Protocol settings recommended by Ventana for urothelial carcinomas for IMFINZI.
5) Protocol settings recommended by Ventana. Analytical claim.

Concentrated antibodies for laboratory developed (LD) assays

mAb **22C3**: 7 of 21 (33%) protocols were assessed as optimal of which two were stained on the Benchmark (Ventana), two on the Omnis (Dako) and one on the Bond III (Leica) platforms.
On the Benchmark XT/Ultra (Ventana), the protocols providing optimal results were based on HIER in CC1 (Ventana) for 48 min., Ab titre of 1:30-40, primary Ab incubation time of 32-64 min. and using OptiView as detection system. Using these protocol settings, 3 of 4 laboratories produced a sufficient staining result.
On Omnis (Dako), the protocols with optimal results were based on HIER in TRS Low pH (Dako) for 40 min., Ab titre of 1:20, primary Ab incubation time of 40-45 min. and using EnVision Flex+ as detection system. Using these protocol settings, 2 of 2 laboratories obtained an optimal staining result.
On Bond III (Leica), one protocol obtained an optimal result, based on HIER in Bond™ Epitope Retrieval Solution 2 for 25 min., Ab titre of 1:10, primary Ab incubation of 30 min. and using Bond Refine as detection system. Only this laboratory used these protocol settings.

Table 4. **Optimal results for PD-L1 for the most commonly used antibody as concentrate on the main IHC systems***

Concentrated antibodies	**Ventana/Roche BenchMark GX/XT/Ultra**		**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Leica Bond III/Max**	
	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**	2/10** (20%)	-	1/2	1/2	-	2/5 (40%)	1/1	-

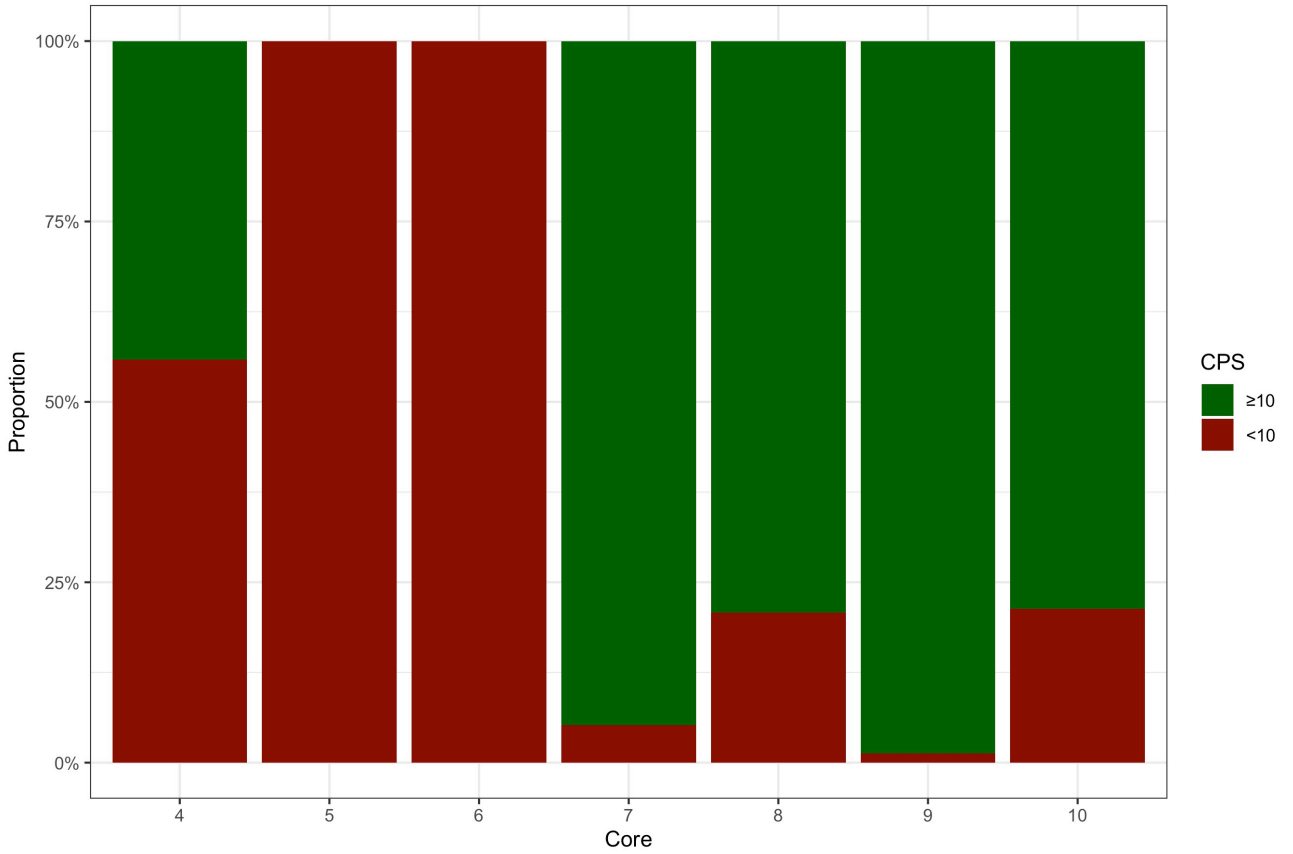
*Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
**number of optimal results/number of laboratories using this buffer

Comments – accuracy of PD-L1 IHC using CPS to guide treatment with KEYTRUDA®
In this first NordiQC run for PD-L1 (uro) in the companion module C5, a pass rate of 76% was observed for the participants performing PD-L1 (uro) IHC assays to identify patients with urothelial carcinomas to be treated with KEYTRUDA® as immune therapy using CPS as scoring method.
Insufficient results were most frequently characterized by a reduced proportion of cells being PD-L1 positive compared to the level expected and defined by the PD-L1 IHC pharmDx assay SK006, Dako. 86% (19 of 22) of the insufficient results were characterized by a too weak or false negative staining reaction in either tumour cells and/or infiltrating lymphocytes/macrophages.
False positive results were seen in 5% (1 of 22) of the insufficient results, and the remaining two insufficient results were caused by poor signal-to-noise ratio and impaired morphology.

The Dako/Agilent 22C3 pharmDx assay SK006, applied by protocol settings in compliance with the vendor recommendations, obtained an overall pass rate of 91% (see table 3). Eight laboratories used the kit off-label on other staining platforms (BenchMark, Ventana, Omnis, Dako and Bond III, Leica) with a total pass rate of 88%. In this context, it must be emphasized that off-label use of approved companion diagnostic assays can provide inaccurate test results and require an extended and often challenging internal validation.
The Ventana PD-L1 IHC assay 790-4905/740-4907, SP263 was the most widely used assay for PD-L1 (uro) and provided an overall pass rate of 93%, when based on protocol settings in compliance with the vendor recommendations (e.g. package insert for urothelial cancers for IMFINZI). When modifying the protocol settings, a decreased pass rate of 78% was seen (see table 3).

Laboratory developed (LD) assays based on concentrated Abs, a "non-companion diagnostic approved" RTU format, or a companion diagnostic assay not used strictly accordingly to the recommended protocol settings provided by the vendor were used by 66% (61 of 93) of the participants and for this group a pass rate of 70% (45 of 64) was observed. mAb clone 22C3 was most widely used concentrate, and when using optimal protocol settings, a pass rate of 74% (14 of 19) was seen. Optimal results were obtained on all the main fully automated IHC platforms from Ventana (BenchMark), Dako (Omnis) and Leica (Bond III). In addition several other Abs as clones CAL10 and ZR3 could be used to generate results evaluated as optimal in this assessment.
However it has to be emphasized that both off-label use of companion diagnostic assays e.g. using a companion diagnostic assay for other intended use as approved claims, modifying the protocol settings for an approved companion diagnostic assay or using a LD assay for PD-L1 status to guide treatment stratification must be meticulously validated by the laboratory.

PD-L1 CPS interpretation and scoring consensus:
Participants were asked to evaluate the CPS in each of the seven urothelial carcinomas included in the assessment material. The overall interpretation of PD-L1 expression and consensus rates of the participants are shown in graph 1.



Graph 1. **NordiQC PD-L1 (uro) run C5: participant interpretation of CPS**

As seen in graph 1, tissue core no. 4 was been most challenging to score CPS for the laboratories. This finding is not surprising and can be related both to the subjectivity and complexity to interpret the CPS. Tissue cores no. 8 and 10 also challenged the laboratories but to lesser extent than tissue core no. 4. All laboratories scored correctly tissue cores no. 5 and 6 as CPS <10, and the majority of the participants scored tissue cores no. 7 and 9 as CPS ≥10.
Reference slides have been made throughout the blocks in the NordiQC reference laboratory and no significant heterogeneity was observed in the urothelial cancers.

Controls
Tonsil and placenta were used as positive and negative tissue controls. In this assessment, tonsil was found to be superior to placenta, as tonsil displayed a dynamic and clinical relevant range of PD-L1 expression levels, whereas placenta virtually only contained cells (trophoblast cells) with high-level PD-L1 expression. Using PD-L1 IHC 28-8 (SK005, Dako/Agilent), 22C3 (GE006 and SK006, Dako/Agilent) or SP263 (790-4905/4907 and 741-4905), Ventana/Roche) and obtaining an optimal staining result, tonsil displayed the following reaction pattern: No staining reaction in the vast majority of lymphocytes including mantle zone and germinal centre B-cells, no staining reaction in superficial epithelial cells, a weak to moderate, typically punctuated membranous staining reaction of the majority of germinal centre macrophages and finally a moderate to strong staining reaction of the majority of epithelial crypt cells. It was observed that SP263 (790-4905/740-4907, Ventana/Roche) provided a higher proportion of positive inter and intra-follicular immune cells compared to the Dako/Agilent PD-L1 assays (SK005, SK006 and GE006).
However, it was observed that a fully acceptable staining pattern in tonsil could be obtained together with an insufficient result in the urothelial cancers. This underlines the need to identify more reliable positive and negative (tissue) controls for PD-L1 and/or improve the interpretation criteria for a sufficient staining reaction in tonsils e.g. more accurately specify number and intensity of cells expected to be demonstrated.

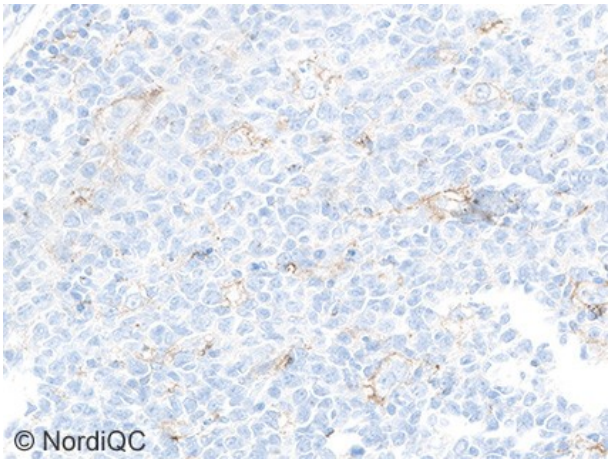


Fig. 1a
Optimal staining result of tonsil (germinal centre) using the pharmDx IHC PD-L1 assay, SK006, Dako/Agilent based on the mAb clone 22C3. Same protocol used in Figs. 2a-6a. Germinal centre macrophages show a weak membranous staining reaction. The vast majority of lymphoid cells are negative.

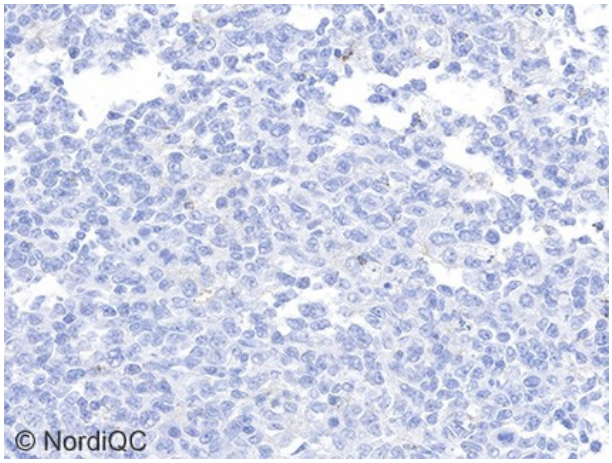


Fig. 1b
Insufficient staining result of tonsil (germinal centre) using the mAb clone 22C3 as concentrate within a laboratory developed assay. The protocol was based on HIER in a low pH buffer and a less sensitive detection system.
Same protocol in Figs. 2b-6b. Virtually all germinal centre macrophages are negative.

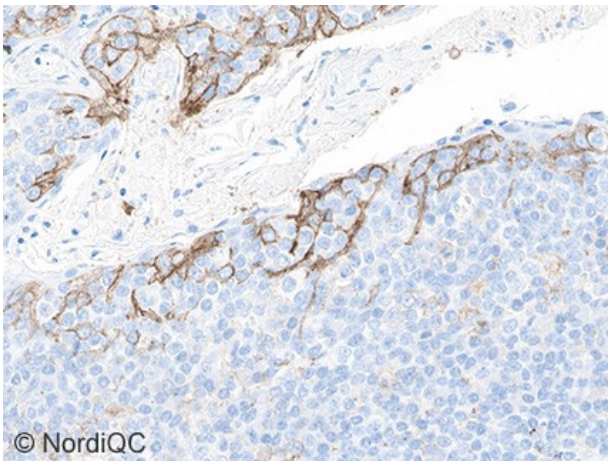


Fig. 2a
Optimal staining result of tonsil (crypt epithelial cells) using same protocol as in Fig. 1a.
A moderate staining reaction is seen in the majority of epithelial crypt cells.

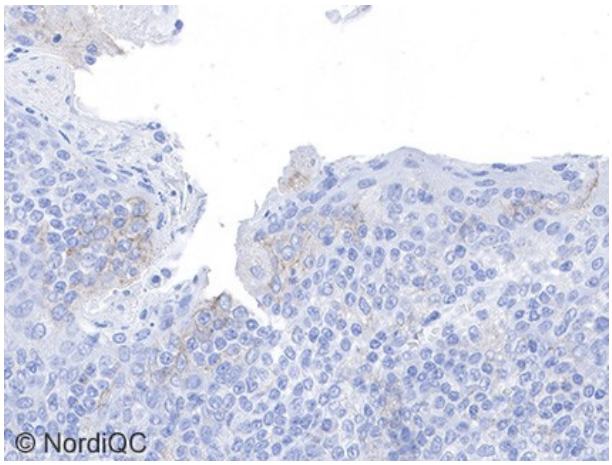


Fig. 2b
Insufficient staining result of tonsil (crypt epithelial cells) using same protocol as in Fig. 1b.
Only few epithelial crypt cells show a too weak and less distinct staining reaction compared to the optimal result shown in Fig. 2a.

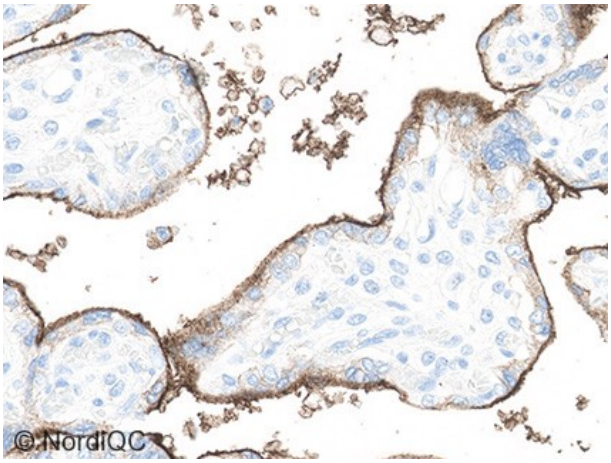


Fig. 3a
Optimal staining result of placenta using same protocol as in Figs. 1a-2a. Trophoblast cells display a moderate to strong predominantly membranous staining reaction.

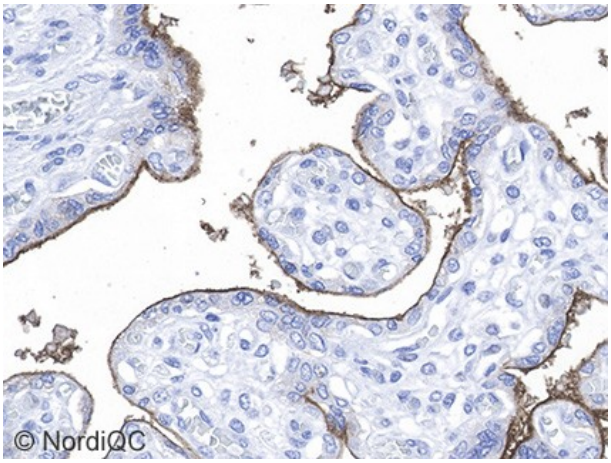


Fig. 3b
Staining result of placenta using same protocol as in Figs. 1b-2b. A weak to moderate staining reaction is seen in the majority of the trophoblast cells.

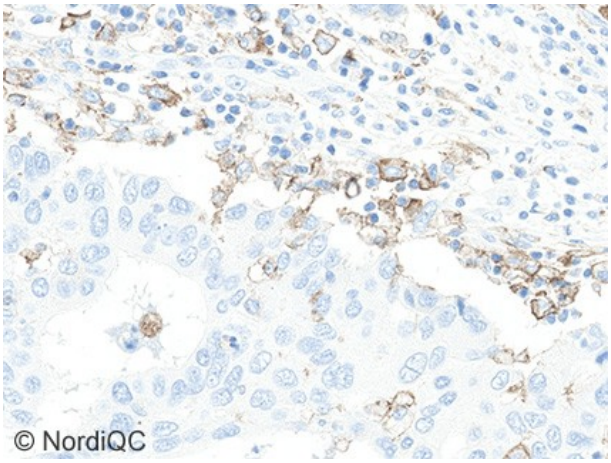


Fig. 4a
Optimal staining result of the urothelial carcinoma tissue core no. 4 using same protocol as in Figs. 1a-3a. All tumour cells are negative. The majority of lymphocytes and macrophages show a moderate staining reaction giving a CPS score ≥10.

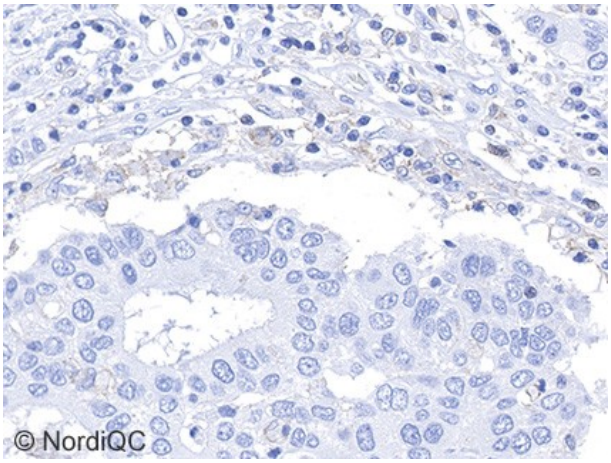


Fig. 4b
Insufficient staining result of the urothelial carcinoma tissue core no. 4, using same protocol as in Figs. 1b-3b. The tumour cells are negative as expected. The proportion of positive lymphocytes and macrophages is significant reduced as only a few scattered cells are found weakly positive, resulting in a CPS score <10.

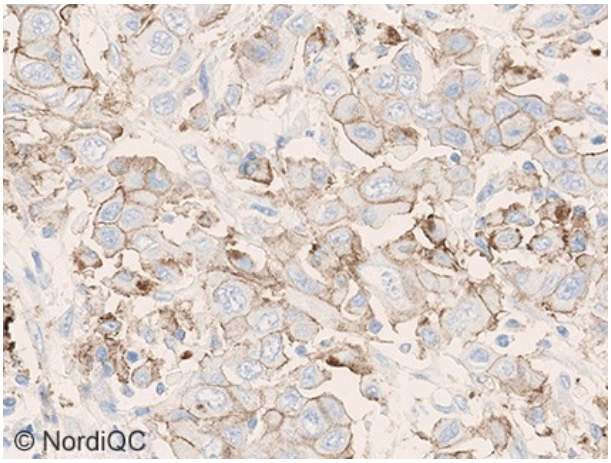


Fig. 5a
Optimal staining reaction of the urothelial carcinoma tissue core no. 7 using same protocol as in Figs. 1a-4a. Virtually all tumour cells display a weak but distinct membranous staining reaction. Lymphocytes and macrophages also show a moderate staining reaction.
The tumour was categorized as CPS ≥10.

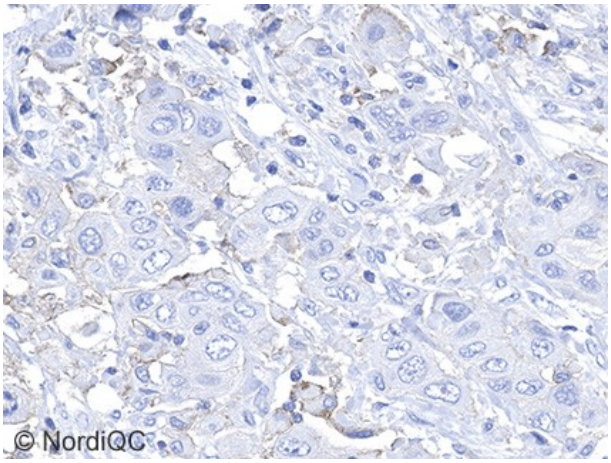


Fig. 5b
Insufficient staining reaction of the urothelial carcinoma tissue core no. 7 using same protocol as in Figs. 1b-4b. The majority of both neoplastic cells, lymphocytes and macrophages are negative providing a CPS <10.
Compare with the optimal result in Fig. 5a, same area.

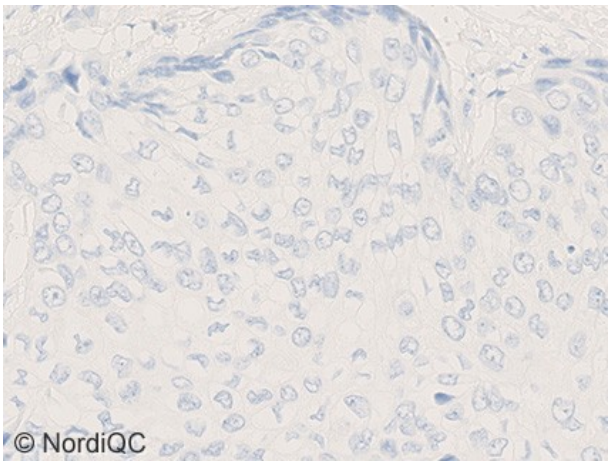


Fig. 6a
Optimal staining result of the urothelial carcinoma tissue core no. 6 using same protocol as in Figs. 1a-5a. No staining reaction is seen in either tumour cells or immune cells, giving a CPS <10.

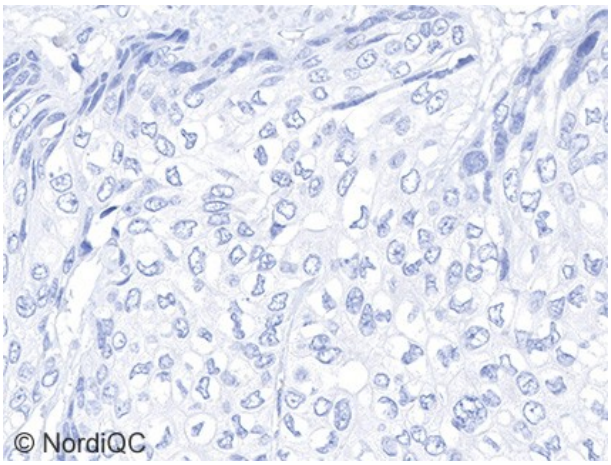


Fig. 6b
Staining result of the urothelial carcinoma tissue core no. 6 using same protocol as in Figs. 1b-5b. No staining reaction is seen in neither tumour nor immune cells.

HLK/SN/RR/MV 08.07.2019