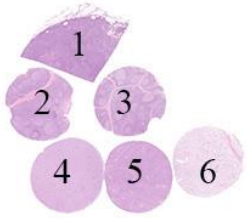
Assessment Run 66 2022
CD10

Purpose
Evaluation of the technical performance and the level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD10, discriminating Diffuse Large B-Cell Lymphoma (DLBCL) of Germinal centre B-cell subtype (GCB) from non-GCB subtype and identifying clear cell renal cell carcinoma (ccRCC) in the characterization of tumours of unknown origin. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for CD10 (see below). Cases diagnosed with DLBCL were classified according to Hans[1] algorithm in which neoplastic B-cells of the GCB phenotype is characterized being CD10 positive or present with the phenotype CD10 neg., BCL6 pos., and MUM1 neg. A cut-off value of ≥30% positive neoplastic B-cells was applied for each individual marker.
[1]Hans CP, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275-82.

Material
The slide to be stained for CD10 comprised:

1. Follicular lymphoma, 2-3. Tonsil, 4. DLBCL (non-GCB subtype), 5. DLBCL (GCB subtype), 6. Clear cell renal cell carcinoma.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD10 staining as optimal included:

- A moderate to strong, distinct membranous staining reaction of virtually all germinal centre B-cells and scattered stromal cells in tonsils.
- An at least moderate, distinct membranous staining reaction of virtually all neoplastic B-cells in the Follicular lymphoma.
- At least weak to moderate, distinct membranous staining reaction of ≥30% of the neoplastic B-cells in the DLBCL (GCB subtype).
- A moderate to strong, distinct membranous staining reaction of virtually all neoplastic cells in the ccRCC.
- An at least weak to moderate, distinct staining reaction of neutrophil granulocytes in all the specimens.
- No staining reaction of the neoplastic B-cells in the DLBCL (non-GCB subtype), mantle zone B-cells and squamous epithelial cells of the tonsil.

Participation

Number of laboratories registered for CD10, run 66	417
Number of laboratories returning slides	393 (94%)

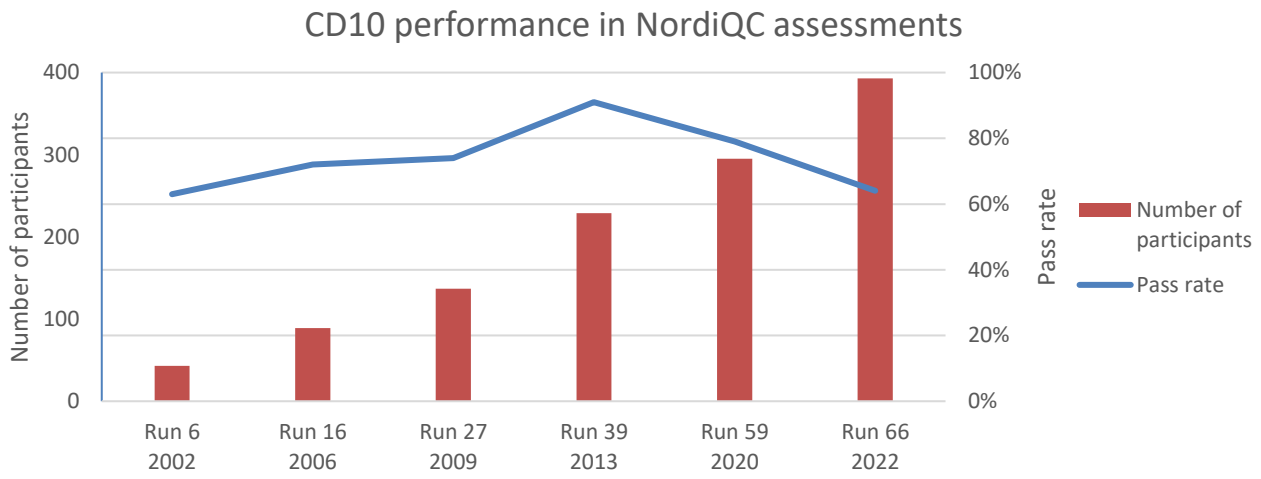
Results
At the date of assessment, 94% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

393 laboratories participated in this assessment and 64% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Inefficient Heat Induced Epitope Retrieval (HIER) – too short time or use or acidic buffer.
- Too low concentration of the primary antibody or too short incubation time.
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters.
- Less successful performance of the mAb clone 56C6 from Cell Marque and the ready-to-use (RTU) system 790-4506 (Ventana/Roche) based on the rmAb clone SP67.
- Unexplained technical issues.

Performance history
This was the sixth NordiQC assessment of CD10. The pass rate has decreased significantly compared to the results obtained in the previous two runs (see Graph 1), which primarily is due to the increased usage of less successful RTU systems (see Table 1) and the composition of the assessment material being more challenging but representing the diagnostic usage and cut-off thresholds for DLBLCs.

Graph 1. **Proportion of sufficient results for CD10 in the six NordiQC runs performed**



Conclusion
The mAb clones **56C6**, **MX002**, **UMAB235**, **DAK-CD10**, **GM106** and rmAb clones **QR021**, **SP67** could all produce optimal results for CD10. The concentrated format of the mAb clone 56C6 provided an optimal result on all the main fully automated IHC platforms (Ventana/Roche, Dako/Agilent and Leica Biosystems), however the mAb clone 56C6 proved to be more challenging on the Ventana Benchmark Ultra. The corresponding RTU systems based on the mAb clones 56C6 from Dako/Agilent and Leica Biosystems as well as the RTU system based on the mAb clone DAK-CD10 (Dako/Agilent) were the most successful assays. Pooled together, the pass rate of laboratories using aforementioned RTU systems was 87% with 57% being optimal. The RTU system based on rmAb clone SP67 (Ventana/Roche) was used by 32% of all participants and provided a very low pass rate of 29%, 4% optimal.
Tonsil is recommended as positive and negative tissue control for CD10. Virtually all the germinal centre B-cells must show an at least moderate but distinct membranous staining reaction, whereas mantle zone B-cells and squamous epithelial cells must be negative. Scattered stromal cells and neutrophil granulocytes must display an at least weak membranous/cytoplasmic staining reaction.

Table 1. **Antibodies and assessment marks for CD10, Run 66**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **56C6**	62	Leica/Novocastra	29	15	15	3	71%	46%
mAb clone **56C6**	6	Cell Marque	1	0	3	2	17%	17%
mAb clone **56C6**	4	Biocare Medical	2	2	0	0	-	-
mAb clone **56C6**	2	Dako/Agilent	1	0	1	0	-	-
mAb clone **56C6**	3	Monosan/Sanbio	2	1	0	0	-	-
mAb clone **56C6**	2	Thermo Scientific/Epredia	1	1	0	0	-	-
mAb clone **56C6**	1	Zytomed	0	1	0	0	-	-
mAb clone **56C6**	1	Immunologic	1	0	0	0	-	-
mAb clone **56C6**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **MX002**	1	Fuzhou Maixin Biotech	1	0	0	0	-	-
mAb clone **IHC525**	1	GenomeMe	0	1	0	0	-	-
mAb clone **UMAB235**	4	ZSBio	2	2	0	0	-	-
rmAb clone **BP6059**	1	Biolynx	0	1	0	0	-	-
rmAb clone **EP195**	1	Quartett	0	0	1	0	-	-
Conc total	90		41	24	20	5	72%	46%

Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **56C6 GA648 (VRPS)**[3]	24	Dako/Agilent	17	4	3	0	88%	71%
mAb clone **56C6 GA648 (LMPS)**[4]	26	Dako/Agilent	20	4	2	0	92%	77%
mAb clone **56C6 IR/IS648 (VRPS)**[3]	4	Dako/Agilent	0	1	3	0	-	-
mAb clone **56C6 IR/IS648 (LMPS)**[4]	32	Dako/Agilent	17	9	6	0	81%	53%
mAb clone **56C6 PA0270/0131 (VRPS)**[3]	19	Leica Biosystems	17	2	0	0	100%	89%
mAb clone **56C6 PA0270/0131 (LMPS)**[4]	21	Leica Biosystems	10	8	3	0	86%	48%
mAb clone **56C6 MAD-002022QD**	3	Master Diagnostica	2	0	0	1	-	-
mAb clone **56C6 110M-10/17/18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **56C6 OPAI 129 T60**	1	Biocare Medical	0	0	0	1	-	-
mAb clone **56C6 AM451**	1	BioGenex	0	0	0	1	-	-
mAb clone **56C6 2-CD052**	1	Quartett	1	0	0	0	-	-
mAb clone **DAK-CD10 GA786 (VRPS)**[3]	18	Dako/Agilent	3	13	2	0	89%	17%
mAb clone **DAK-CD10 GA786 (LMPS)**[4]	17	Dako/Agilent	8	8	1	0	94%	47%
mAb clone **DAK-CD10 IR786/ IS786 (VRPS)**[3]	1	Dako/Agilent	1	0	0	0	-	-
mAb clone **DAK-CD10 IR786/ IS786 (LMPS)**[4]	2	Dako/Agilent	0	1	1	0	-	-
mAb clone **C6D1 CCM-0391**	1	Celnovte Biotechnology	0	1	0	0	-	-
mAb clone **MX002 MAB-0668**	1	Fuzhou Maixin Biotech	1	0	0	0	-	-
mAb clone **GM106 GT2004**	1	Gene Tech	1	0	0	0	-	-
rmAb clone **QR021 8386-C010**	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone **SP67 790-4506 (VRPS)**[3]	16	Ventana/Roche	3	4	9	0	44%	19%
rmAb clone **SP67 790-4506 (LMPS)**[4]	110	Ventana/Roche	2	27	75	6	26%	2%
rmAb clone **521I3K1**	1	Abcarta	0	0	1	0	-	-
rmAb clone **MyM1-CD10 unknown**	1	Zybio	0	1	0	0	-	-
RTU total	303		104	83	107	9	62%	34%
Total	393		145	107	127	14		
Proportion			37%	27%	32%	4%	64%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of CD10, Run 66
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **56C6**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (CC1, Ventana/Roche) (28/50)*, Target Retrieval Solution (TRS) pH 9 (Dako/Agilent) (3/4), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (7/18). The mAb was typically diluted in the range of 1:10– 1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings, 52 of 70 (74%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **UMAB235**: Protocols with optimal results were all based on HIER using an alkaline buffer pH 9.0 (Ultra-EDTA, Origene) (2/4). The mAb was diluted 1:200 and UltraPATH Plus DAB with linker (Origene) was used as detection system. Using these protocol settings, 4 of 4 (100%) laboratories produced a sufficient staining (optimal or good).

Table 2. **Proportion of optimal results for CD10 for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **56C6**	0/2**	0/1	3/4 (75%)	-	28/50 (56%)	-	7/17 (41%)	0/1

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

Ready-To-Use antibodies and corresponding systems
mAb clone **56C6,** product no. **GA648,** Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 30 min. at 97°C), 12-20 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823 + GV821) as detection system. Using these protocol settings, 30 of 33 (88%) laboratories produced a sufficient result. Four laboratories used the RTU format off-label (deviant platforms).

mAb clone **56C6,** product no. **IR/IS648,** Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 15-20 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision Flex+ (K8000/K8002) as detection system. Using these protocol settings, 12 of 13 (92%) laboratories produced a sufficient result. 16% (4/25) of the laboratories applied VRPS of which none provided an optimal result (see Tables 1 and 4). 11 laboratories used the RTU format off-label (e.g. deviant platforms).

mAb clone **56C6,** product no. **PA0270/0131,** Leica Biosystems, BOND III/BOND MAX:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20-60 min. at 92-100°C), 15-30 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 30 of 31 (97%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 100% (19/19) and 89% (17/19) of were optimal (see Tables 1 and 4). Five laboratories used the RTU format off-label (e.g. deviant platforms).

rmAb clone **SP67**, product no. **790-4506**, Ventana/Roche, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 92 min. at 95-100°C), 20 or 28 min. incubation of the primary Ab and UltraView with amplification (760-500/760-080) or OptiView with or without amplification (760-700/760-099) as detection systems. Using these protocol settings, 8 of 16 (50%) laboratories produced a sufficient result. 13% (16/126) of the laboratories applied VRPS of which 3 provided an optimal result (see Tables 1 and 3). One laboratory used the RTU format off-label (deviant platform).

mAb clone **DAK-CD10,** product no. **GA786,** Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 30 min. at 97-99°C), 12-20 min. incubation of the primary Ab and EnVision FLEX with or without Linker (GV800/GV823 + GV821) as detection system. Using these protocol settings, 28 of 31 (90%) laboratories produced a sufficient result. All laboratories (11/11) using the EnVision FLEX+ as a detection system produced a sufficient result. Applying vendor recommended protocol settings (VRPS), the proportion of

sufficient results was 89% (16/18) and 17% (3/18) were optimal (see Tables 1 and 4). One laboratory used the RTU format off-label (deviant platform).

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 according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for CD10 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako Omnis mAb **56C6 GA648**	88% (21/24)	71% (17/24)	90% (19/21)	76% (16/21)
Dako AS mAb **56C6 IR648**	25% (1/4)	0% (0/4)	76% (16/21)	52% (11/21)
Leica Bond III/Max mAb **56C6 PA270/0131**	100% (19/19)	89% (17/19)	81% (13/16)	50% (8/16)
Dako Omnis mAb **DAK-CD10 GA786**	89% (16/18)	17% (3/18)	94% (15/16)	44% (7/16)
Dako AS mAb **DAK-CD10 IR786**	100% (1/1)	100% (1/1)	50% (1/2)	50% (1/2)
VMS Ultra/XT/GX rmAb **SP67 790-4506**	44% (7/16)	19% (3/16)	26% (29/109)	2% (2/109)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment of CD10, the prevalent feature of an insufficient result was either a too weak or false negative staining reaction of cells expected to be demonstrated, which was seen in 80% (114/142) of the insufficient results. The remaining 20% of the insufficient results were mainly characterized by poor signal-to-noise ratio, false positive, imprecise membranous staining reaction or a granular staining reaction interfering interpretation. Virtually all the participating laboratories were able to demonstrate CD10 in the neoplastic cells of the ccRCC, whereas the DLBCL (GCB subtype), follicular lymphoma and tonsils were more challenging and required an optimally calibrated IHC system. The majority of laboratories who received an insufficient result were unable to demonstrate a distinct complete membranous staining reaction of the expected cells to be positive and could only provide a general too weak and diffuse imprecise staining reaction. False positivity was primarily seen in the non-GCB subtype DLBCL using the RTU product 790-4506 (Ventana/Roche) based on the rmAb SP67 in combination with OptiView with amplification as detection system.

23% (90/393) of the participants used Abs as concentrated formats within laboratory developed (LD) assays for CD10 with 72% (65/90) producing a sufficient result, 46% (41/90) optimal. Within these 91% (82/90) used the mAb clone 56C6, most commonly from Leica Biosystems. Optimal results could be obtained on all main automated staining platforms from Ventana/Roche, Dako/Agilent and Leica Biosystems, however a variation in pass rates were observed especially between the visualization systems used, but also related to the origin/vendor of the mAb clone 56C3 – see Table 1.
The most widely used visualization system within the participants who used the mAb clone 56C6 as a concentrate was OptiView with or without amplification (760-700/760-099). Overall, 51% (42/82) of laboratories used this system of which 76% (32/42) obtained a sufficient result, 60% (25/42) optimal. Out of the 10 insufficient results, 50% used the mAb clone 56C6 concentrate produced by Cell Marque (110M-14/15/16, dilution factor between 1:20-1:50) and 3 of the other 5 laboratories used Optiview with amplification. When excluding the Cell Marque product and protocols based on OptiView with amplification, the pass rate for protocols based on OptiView as the visualization system was 93% (26/28), 79% (22/28) optimal (Ab typically diluted in the range of 1:10-1:50).
UltraView with amplification was less successful, as only a pass rate of 33% (3/9) was obtained despite using similar protocol settings compared to OptiView e.g. regarding titre range of primary Ab and HIER. All three participants with a sufficient result obtained an optimal mark with HIER for 64 min. in Ultra CC1

(950-224), antibody dilution range 1:10 (PBS based diluent by Ventana/Roche, 251-018) and 1:25 (TBS based diluent by Dako/Agilent, S3022), antibody incubation for 32 min.
Laboratories using Bond Refine DS9800 (Leica Biosystems) as visualization system obtained a pass rate of 83% (14/16), 38% (6/16) optimal (Ab typically diluted in the range of 1:25-1:50).
Laboratories using the Envision FLEX detection system with or without linker (K8000/K8002, GV800/GV823 + GV821, Dako/Agilent) had a pass rate of 75% (6/8), 38% (3/8) optimal (Ab typically diluted in the range of 1:25-1:50).

Although the data is limited, it must be emphasized that all parameters must be optimized and carefully calibrated to provide an IHC protocol that is able to demonstrate CD10 in cellular structures with both low- and high-level of expression in order to accomplish the purpose of the test, as in this assessment is the classification and subtyping of DLBCL providing both prognostic and predictive information.

The majority of participants used a ready-to-use (RTU) system to detect CD10, amounting to 77% (309/393) of all results. In this assessment, the RTU systems developed for and stained on the intended fully automated stainer platforms based on the mAb clone 56C6 by Leica Biosystems (PA0270/0131, BOND) and Dako/Agilent (GA648, Omnis) as well as the mAb clone DAK-CD10 by Dako/Agilent (GA786, Omnis) clearly provided the highest pass rate of 90% (103/114) sufficient and 60% (68/114) optimal results, irrespective of the protocol applied.

The Leica Biosystems RTU system was most successful as all (19/19) laboratories using PA0270/0131 per vendor recommendations provided a sufficient result, with 89% optimal (17/19).

The pass rate among participants using the Dako/Agilent RTU system based on the mAb clone 56C6 (GA648) on the Omnis platform with vendor recommended protocol settings (VRPS) and laboratory modified protocol settings (LMPS) grouped together was 89% (40/45), 73% (33/45) optimal (no differences seen for VRPS and LMPS, see Table 3). Typically, concurring factors caused the insufficient results as excessive counterstain interfering interpretation, the use of non-alkaline HIER buffer and/or unexplained technical issues. The most common deviation of VRPS was a change of the incubation time in primary antibody (17/21), which did not seem to affect the staining quality, assuring the robustness of the product. Five participants used the GA648 product on deviant platforms (Dako Autostainer Link 48+, Leica BOND III, Ventana Benchmark Ultra) of which 100% produced a sufficient result, 80% (4/5) optimal.

36 laboratories used the Dako/Agilent RTU system based on mAb 56C6 for Dako Autostainer (IR648) of which 69% (25/36) used it on the intended staining platform. Only four participants used the VRPS giving a pass rate of 25%. The vast majority (21/25) of laboratories using IR648 on the intended Dako Autostainer modified the protocol, as 76% (16/21) added a linker step to the detection system used. 94% (15/16) provided a sufficient result, 69% optimal. 10 laboratories used the IR648 product on deviant platforms (Leica BOND III, Ventana Benchmark XT/Ultra), 90% (9/10) produced a sufficient result, 50% (5/10) optimal. The only insufficient result was caused by a technical issue.

The new Dako/Agilent RTU system based on the clone DAK-CD10 (GA786) for Dako Omnis was used by 34 participants on the intended staining platform and provided an overall high pass rate of 91% (31/34), 29% (10/34) optimal. 53% (18/34) of the laboratories applied the VRPS with 89% (16/18) receiving a sufficient result, however only 17% (3/18) being optimal. Similar to IR648, mAb 56C6, the most successful modification to the staining protocol was adding a linker step to the visualization system. All laboratories (12/12) using 3-layer visualization system together with HIER in alkaline buffer provided a sufficient result, 50% (6/12) optimal.

In general, it must be emphasized that modifications of vendor recommended protocol settings for the RTU systems including migration of the RTU Abs to another platform than the intended, require a meticulous validation process for the end-users. As seen in this and previous assessments, modifications can be very successful but may also generate sub-optimal or aberrant results and therefore must be carefully monitored.

In this assessment, the most widely used (126/393) and at the same time the least successful RTU system was the Ventana/Roche 790-4506 based on the rmAb clone SP67. Compared to the previous CD10 assessment (run 59, 2020) the overall pass rate has declined from 59% (51/87) to 29% (36/126) which in part can be caused by the more challenging composition of the tissue micro-array (TMA) circulated in this run, however carefully selected to represent the diagnostic usage and cut-off thresholds for DLBLCs and hereby highlighting the technical difficulties laboratories are facing with the product. In line with the results from run 59, no specific parameters causing the low pass rate could be identified as the same protocol settings produced different results. The registered protocol settings (92 min. HIER in CC1, Ab

incubation of 20 or 28 min., UltraView with amplification or OptiView with or without amplification as detection system) provided an optimal result for 5 participants, however only an overall pass rate of 46% (6/13) was obtained indicating that the majority of laboratories could not reproduce the same result. 80% (72/90) of the insufficient results with the rmAb clone SP67 was characterized by a generally too weak and/or imprecise or completely false negative staining reaction. It was observed that many laboratories used the highly sensitive detection system OptiView together with amplification, but with limited success causing false positive staining in the DLBCL non-GCB-subtype and an overall granular imprecise staining reaction, known to be one of the caveats of using the tyramide based amplification system, interfering interpretation.

The sixth assessment of CD10 provided an overall pass rate of 64% which is significantly lower compared to the previous run in 2020 showing a pass rate of 79%. In this assessment, the proportion of laboratories using a concentrated format of CD10 has reduced from 31% to 23%.
The RTU systems based on mAb 56C6 (Leica Biosystems and Dako/Agilent) and the new mAb clone DAK-CD10 (Dako/Agilent) showed a very high pass rate either with the VRPS (88% sufficient, 58% optimal) or LMPS (87% sufficient, 56% optimal), proving the robustness of the products. However, the Ventana/Roche RTU system based on rmAb clone SP67 proved to be technically challenging for the laboratories, providing an inferior pass rate of 29% (36/126) with no reliable protocol settings that could be used to produce a consistent result. It is important to emphasize that laboratories should use a robust Ab, calibrate the protocols correctly and verify/validate the results according to the diagnostic use and expected antigen level of the recommended tissue control materials (see below).

Controls
Tonsil is recommended as positive and negative tissue control for CD10. Virtually all the germinal centre B-cells must show an at least moderate but distinct membranous staining reaction, which should be identifiable even at low power magnification (4x) – see Figs. 1a and 1b. It must be emphasized that the individual germinal centre B-cells must be clearly outlined showing the contours of the membranes. The mantle zone B-cells and squamous epithelial cells must be negative. Scattered stromal cells and neutrophil granulocytes must display an at least weak staining reaction.

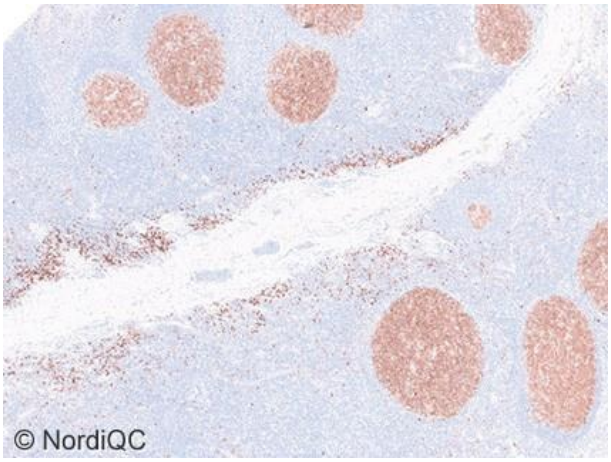


Fig. 1a (x25)
Optimal staining reaction for CD10 in tonsil using the mAb clone DAK-CD10 (RTU GA786 Dako/Agilent) per vendor recommended protocol settings for the Dako Omnis stainer platform seen by a low power magnification. All germinal centre B-cells and scattered neutrophil granulocytes show a moderate to strong distinct membranous staining reaction whereas the mantle zone B-cells are negative. A weak to moderate staining reaction can be seen in the scattered stromal cells. Same protocol used in Figs. 2a-4a.

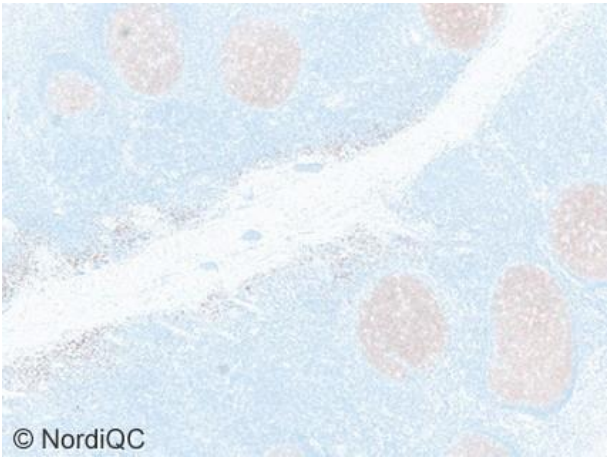


Fig. 1b (x25)
Insufficient staining reaction for CD10 in the tonsil using mAb clone 56C6 as a concentrate (1:25) from Cell Marque, HIER in CC1 (72 min.) and OptiView as detection system. A weak staining reaction in the germinal centre B-cells is seen and no distinct membranous accentuation can be identified. Many of the expected stromal cells remain negative and only a weak staining reaction can be seen in dispersed neutrophil granulocytes. Same protocol used in Figs. 2b-4b – same field as 1a.

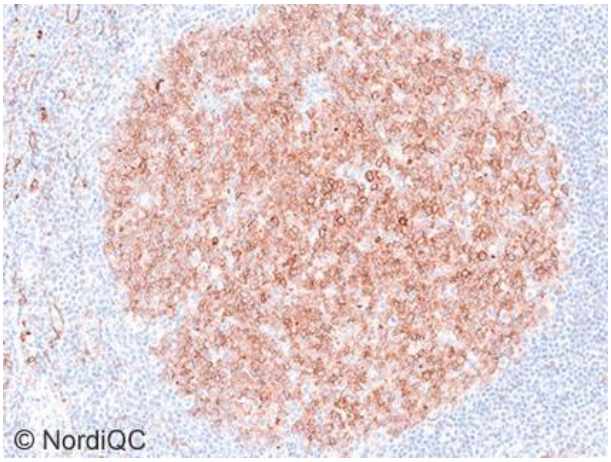


Fig. 2a (x100)
Optimal staining reaction for CD10 in tonsil using same protocol as in Fig. 1a. All germinal centre B-cells show a moderate to strong distinct membranous staining reaction. The mantle zone B-cells are negative. Scattered stromal cells display a weak membranous/cytoplasmic staining reaction. Same protocol used in Figs. 2a-4a.

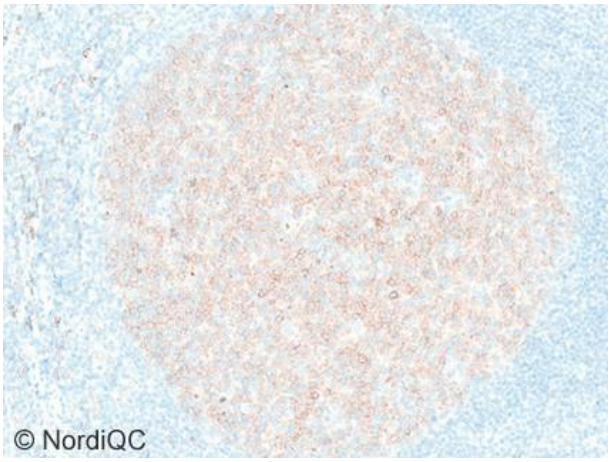


Fig. 2b (x100)
Insufficient staining reaction for CD10 in the tonsil using same protocol as in Fig. 1b. The intensity and proportion of positive germinal centre B-cells has decreased significantly compared to the level expected, displaying mainly a weak granular staining reaction. Also the stroma cells are negative. Same protocol used in Figs. 2b-4b – same field as 1a.

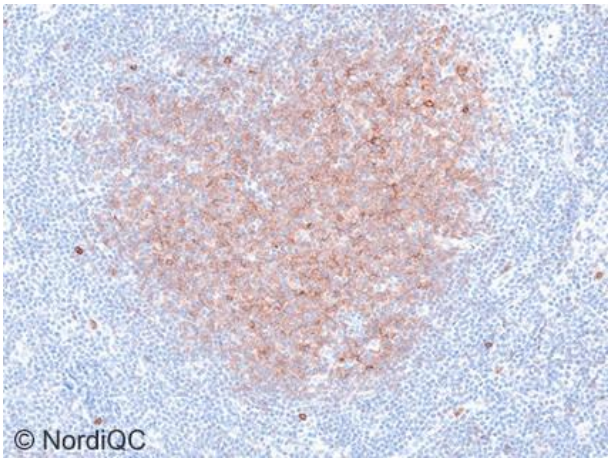


Fig. 3a (x100)
Optimal staining reaction for CD10 in the follicular lymphoma (FL) using same protocol as in Figs. 1a-2a. The majority of the neoplastic B-cells show a moderate to strong, distinct membranous staining reaction.

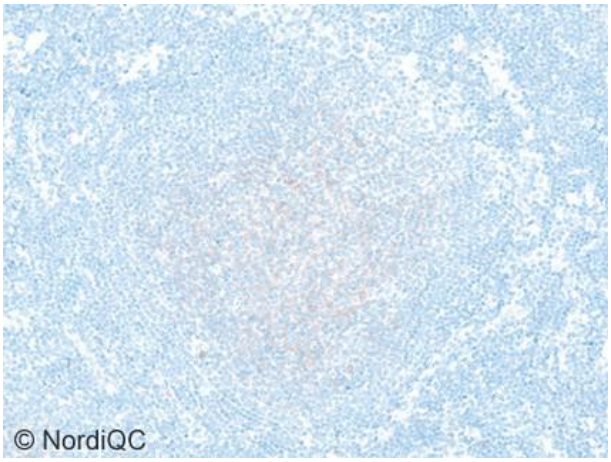


Fig. 3b (x100)
Insufficient staining for CD10 in the FL using same protocol as in Figs. 1b-2b. The neoplastic B-cells only display an indistinct faint or completely false negative staining reaction - compare with Fig. 2a.

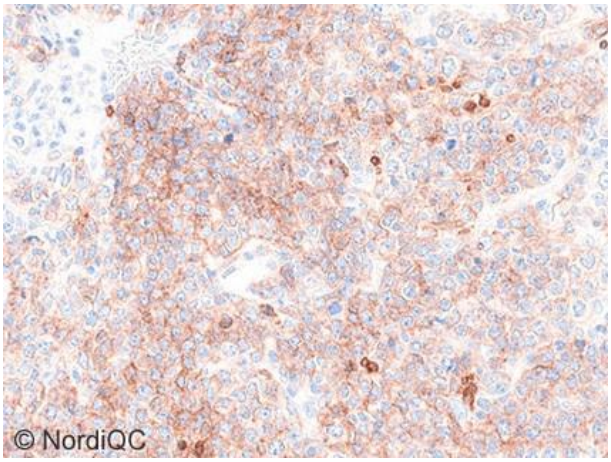


Fig. 4a (x200)
Optimal staining reaction for CD10 in the DLBCL (GCB subtype) using the same protocol as in Figs. 1a-3a. More than 30% of the neoplastic B-cells show a distinct membranous staining reaction.

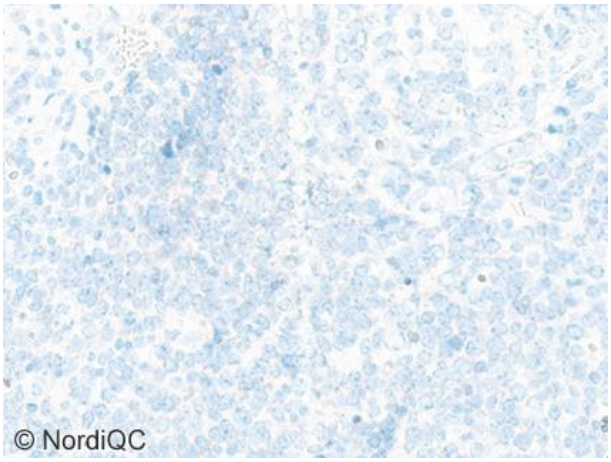


Fig. 4b (x200)
Insufficient staining reaction for CD10 in the DLBCL (GCB subtype) using same protocol as in Figs. 1b-3b. Same field as 3a. Virtually all the neoplastic B-cells are false negative, compromising the diagnostic use in a clinical setting - compare with Fig. 3a.

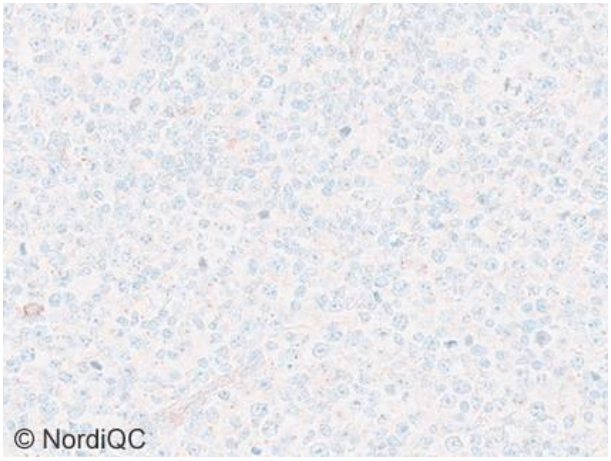


Fig. 5a (x200)
Optimal staining reaction for CD10 in the DLBCL (non-GCB subtype) using the rmAb clone SP67 (RTU 790-4506 Ventana/Roche) per vendor recommended protocol settings using UltraView with amplification. All neoplastic cells are negative however a focal punctuated staining reaction is seen which does not interfere the interpretation in this tissue core.

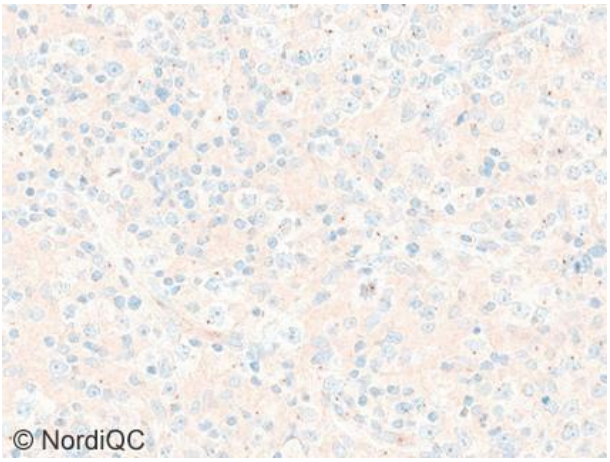


Fig. 5b (x200)
Insufficient staining reaction for CD10 in DLBCL (non-GCB subtype) using the rmAb clone SP67 with the same protocol settings as in Fig. 5a by another participant. The majority of cells show at least weak mainly cytoplasmic but also membranous false positive staining reaction compromising the interpretation – compare with Fig. 5a. Most participants produced an insufficient result using the same protocol settings as were used in laboratories achieving a sufficient mark which highlights the technical difficulties faced with the rmAb clone SP67.

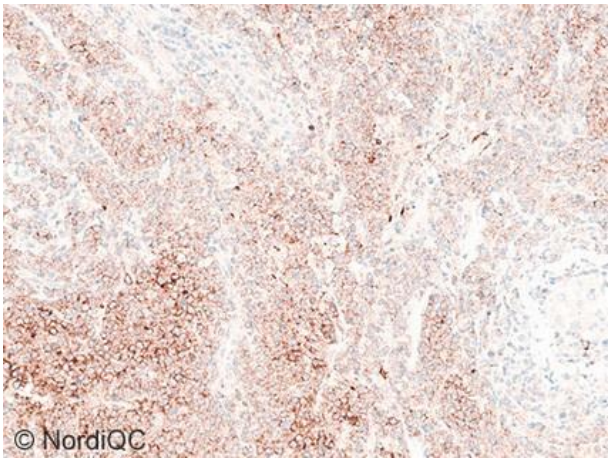


Fig. 6a (x100)
Insufficient staining reaction for CD10 in the DLBCL (GCB subtype) using the rmAb clone SP67 (RTU 790-4506 Ventana/Roche), HIER in CC1 (64 min.) and OptiView with extended incubation in amplification reagents (12+12 min.). In addition to the neoplastic cells showing granular primarily membranous reaction pattern, an unspecific granular staining reaction can be seen in all cells – see Fig. 6b.

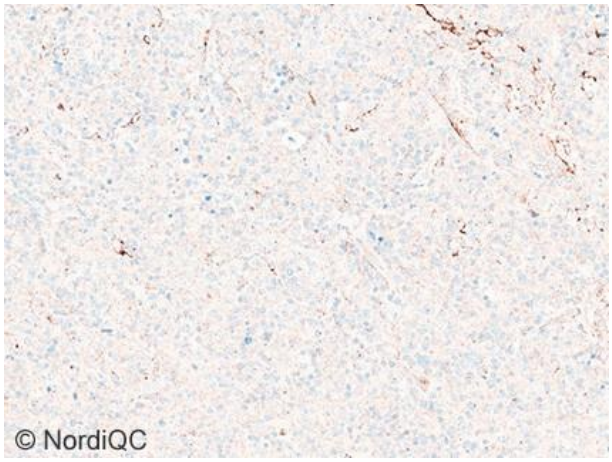


Fig. 6b (x100)
Insufficient staining reaction for CD10 in the (DLBCL non-GCB subtype) using the same protocol settings by the same participant as in Fig. 6a. Unspecific granular staining reaction most likely caused by the tyramide based amplification reagents interferes the interpretation as the granules are most prominent on the membranes of neoplastic cells – compare with sufficient result in Fig. 5a.

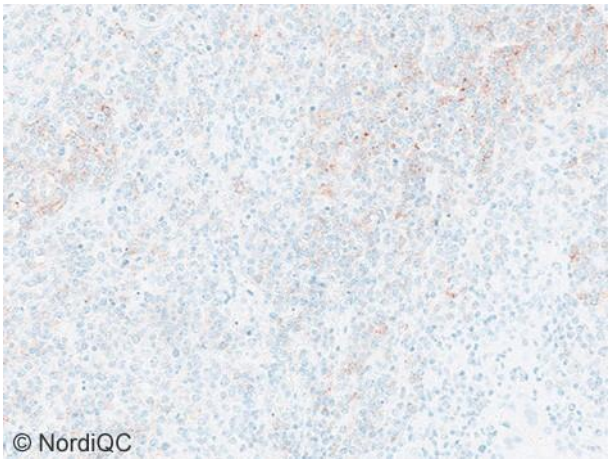


Fig. 7a (x100)
Insufficient staining reaction for CD10 in the DLBCL (GCB subtype) using virtually the same protocol settings as in Figs. 6a-6b using OptiView with incubation in the amplification reagents for 4+4 min. The analytical sensitivity level provided by the assay is too low as the majority of the neoplastic cells are completely negative or only show a granular reaction pattern. Virtually all membranes of malignant B-cells are negative, also indicating the lack of reproducibility of the assay – compare to Fig. 6a.

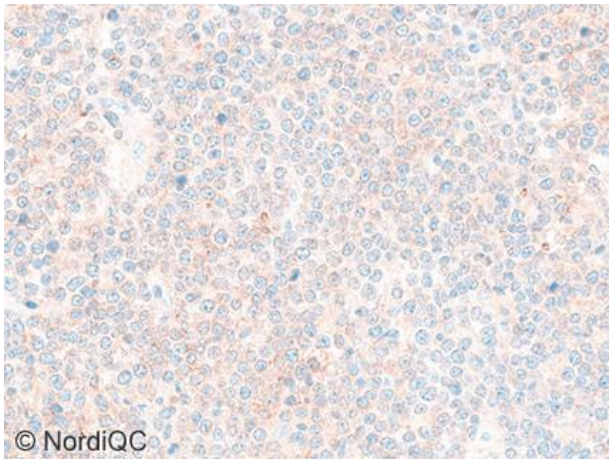


Fig. 7b (x200)
Insufficient staining reaction for CD10 in the DLBCL (GCB subtype) using mAb clone 56C6 (RTU 110M Cell Marque) demonstrating one of the most common causes of an insufficient result. The expected and characteristic membranous accentuation of the neoplastic cells is missing, and only a weak, diffuse and indistinct reaction obtained, hampering the evaluation as it is impossible to differentiate between positive and negative cells. Compare to the optimal result in the same core in Fig. 4a.

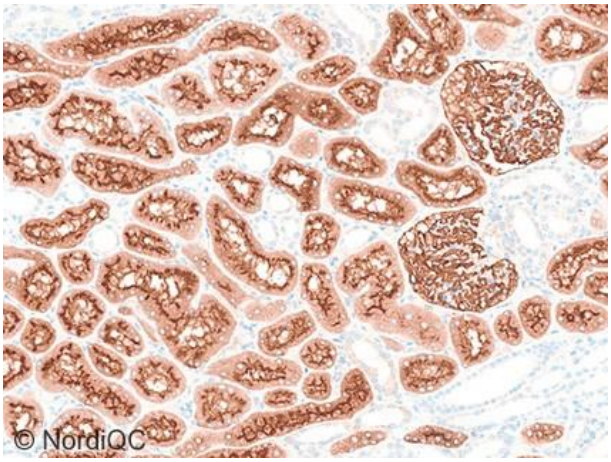


Fig. 8a (x100)
Participant On-slide control of a kidney using same protocol settings as in Figs. 1b-4b. A moderate to strong membranous and cytoplasmic staining of the glomerular epithelium, Bowman's capsule and proximal tubules can still be seen although the analytical sensitivity of the assay is too low as seen on the circulated TMA tissue cores (as in Figs. 1b-4b), indicating that kidney is not a reliable control. Also see Fig. 8b, same protocol on On-slide control of tonsil.

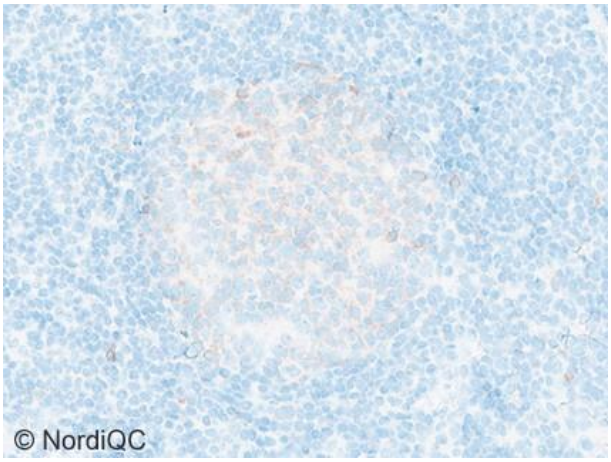


Fig. 8a (x200)
Participant On-slide control of a tonsil used together with the kidney showed in Fig. 8a. A very weak staining reaction can be seen in the germinal centre B-cells, however many of the expected positive cells remain completely negative. Using tonsil as a control is a good indicator of the analytical sensitivity of the CD10 assay – compared to kidney in Fig. 8a. It has to be emphasized that it is of utmost importance that laboratories monitor the expected staining reaction in controls, with appropriate level of the target antigen and act upon it when an insufficient staining reaction is seen.

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