NordiQC

Assessment Run 68 2023
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 of:
1. Clear cell renal cell carcinoma, 2-3. Tonsil, 4. DLBCL (GCB subtype),
5. DLBCL (non-GCB subtype), 6. Follicular lymphoma.

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 an at least weak staining reaction in scattered stromal cells in tonsils.
- A moderate to strong, distinct membranous staining reaction of virtually all neoplastic cells in the ccRCC.
- At least weak to moderate, distinct membranous staining reaction of ≥30% of the neoplastic B-cells in the DLBCL (GCB subtype).
- An at least moderate, distinct staining reaction of neutrophil granulocytes in all the specimens.
- An at least moderate, distinct membranous staining reaction of virtually all neoplastic B-cells in the Follicular lymphoma.
- 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.

*A weak to moderate membranous staining reaction in a minor proportion of neoplastic cells in the DLBCL (non-GCB subtype) in one of the TMAs was accepted due to heterogeneity of the tumor and observed only in one TMA.

Participation

Number of laboratories registered for CD10, run 68	433
Number of laboratories returning slides	403 (93%)

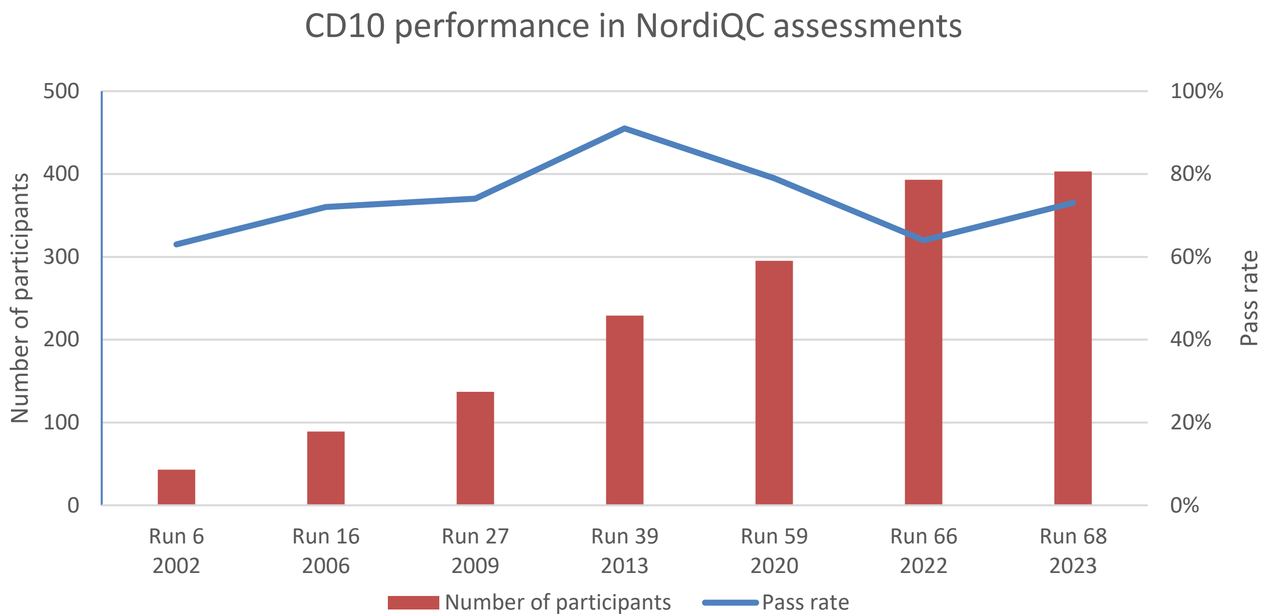
Results
At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

403 laboratories participated in this assessment and 73% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).

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

Performance history
This was the seventh NordiQC assessment of CD10. The overall pass rate has increased compared to the results obtained in the previous run (see Graph 1).

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



Conclusion
The mAb clones **56C6**, **DAK-CD10, MX002** and rmAb clone **SP67** could all produce optimal results for CD10. The widely used concentrated format of the mAb clone 56C6 from Leica Biosystems provided an optimal result on all the main fully automated IHC platforms (Ventana/Roche, Dako/Agilent and Leica Biosystems). 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 60% being optimal. The RTU system based on rmAb clone SP67 (Ventana/Roche) was used by 33% of all participants and provided a pass rate of 59%, 25% 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 68**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **56C6**	58	Leica Biosystems	38	7	9	4	78%	66%
mAb clone **56C6**	7	Cell Marque	1	0	6	0	14%	14%
mAb clone **56C6**	3	Monosan/Sanbio	3	0	0	0	-	-
mAb clone **56C6**	2	Biocare Medical	2	0	0	0	-	-
mAb clone **56C6**	2	Thermo Scientific/Epredia	1	0	1	0	-	-
mAb clone **56C6**	1	Immunologic	1	0	0	0	-	-
mAb clone **56C6**	1	Zytomed	0	0	1	0	-	-
mAb clone **UMAB235**	1	Unknown	0	1	0	0	-	-
rmAb clone **QR021**	1	Quartett	0	0	1	0	-	-
rmAb clone **ZR329**	1	Zeta Corporation	0	0	0	1	-	-
Conc total	77		46	8	18	5	70%	60%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **56C6** **GA648 (VRPS)**[3]	14	Dako/Agilent	11	3	0	0	100%	79%
mAb clone **56C6** **GA648 (LMPS)**[4]	18	Dako/Agilent	13	3	2	0	89%	72%
mAb clone **56C6** **IR/IS648 (VRPS)**[3]	3	Dako/Agilent	0	2	1	0	-	-
mAb clone **56C6** **IR/IS648 (LMPS)**[4]	13	Dako/Agilent	9	2	2	0	85%	69%
mAb clone **56C6** **PA0270/0131 (VRPS)**[3]	22	Leica Biosystems	12	6	4	0	82%	55%
mAb clone **56C6** **PA0270/0131 (LMPS)**[4]	27	Leica Biosystems	16	3	8	0	70%	59%
mAb clone **56C6** **MAD-002022QD**	4	Master Diagnostica	1	2	1	0	-	-
mAb clone **56C6** **PM 129 AA**	2	Biocare Medical	0	0	1	1	-	-
mAb clone **56C6** **AM451**	1	BioGenex	0	0	0	1	-	-
mAb clone **56C6** **110M-10/17/18/19**	1	Cell Marque	0	0	1	0	-	-
mAb clone **DAK-CD10** **GA786 (VRPS)**[3]	22	Dako/Agilent	12	10	0	0	100%	55%
mAb clone **DAK-CD10** **GA786 (LMPS)**[4]	33	Dako/Agilent	21	11	1	0	97%	64%
mAb clone **DAK-CD10** **IR786 (VRPS)**[3]	5	Dako/Agilent	2	2	0	1	80%	40%
mAb clone **DAK-CD10** **IR786 (LMPS)**[4]	15	Dako/Agilent	7	5	2	1	80%	47%
mAb clone **MX002** **MAB-0668**	2	Fuzhou Maixin Biotech	2	0	0	0	-	-
mAb clone **BY052** **BFM-0348**	1	Bioin Biotechnology	1	0	0	0	-	-
mAb clone **C6D1** **CCM-0391**	1	Celnovte Biotechnology	1	0	0	0	-	-
mAb clone **GM106** **GT2004**	1	Gene Tech	1	0	0	0	-	-
mAb clone **H3C3** **DTBL0204001**	1	DaTe Bioengineering Technology	1	0	0	0	-	-
rmAb clone **SP67** **790-4506 (VRPS)**[3]	18	Ventana/Roche	3	4	10	1	39%	17%
rmAb clone **SP67** **790-4506 (LMPS)**[4]	115	Ventana/Roche	30	41	44	0	62%	26%
rmAb clone **521I3K1** **PA489**	1	Abcarta	0	0	1	0	-	-

rmAb clone **BP6059** **I10262E**	2	Biolynx	1	1	0	0	-	-
rmAb clone **MyM1-CD10** **unknown**	1	Zybio	1	0	0	0	-	-
rmAb clone **DGR020** **DGR020/02022092931RA**	1	Shanghai DG Diagnology Tec	0	0	1	0	-	-
rmAb clone **QR021** **P-C006-70**	1	Quartett	1	0	0	0	-	-
rmAb clone **QR021** **8386-C010**	1	Sakura Finetek	1	0	0	0	-	-
RTU total	326		147	95	79	5	74%	45%
Total	403		193	103	97	10		
Proportion			48%	26%	24%	2%	73%	

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

Detailed analysis of CD10, Run 68

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) (35/47)*, Target Retrieval Solution (TRS) pH 9 (Dako/Agilent) (4/7) and Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (7/14). The mAb was typically diluted in the range of 1:10–1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 52 of 68 (76%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

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 GX/XT/Ultra**		**Leica Biosystems Bond III**	
	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**	2/3**	-	2/4	-	35/47 (74%)	-	7/14 (50%)	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), 12-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823 + GV821) as detection system. Using these protocol settings, 23 of 23 (100%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was also 100% (14/14) and 79% (11/14) provided an optimal result (see Tables 1 and 3).
Four laboratories used the RTU format off-label (deviant platforms).

mAb clone **56C6,** product no. **IR/IS648,** Dako/Agilent, Autostainer+/Autostainer Link 48:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 95-97°C), 20 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002 + K8021) as detection system. Using these protocol settings, 4 of 6 (67%) laboratories produced a sufficient result. 19% (3/16) of the laboratories applied VRPS of which none provided an optimal result (see Tables 1 and 3). *Six laboratories used the RTU format off-label (e.g. deviant platforms or manual staining).*

mAb clone **56C6,** product no. **PA0270/0131,** Leica Biosystems, BOND III/MAX/PRIME:
Protocols with optimal results were typically based on HIER using BERS2 or Bond-Prime Epitope Retrieval Solution 2 (efficient heating time 20-60 min. at 97-100°C), 15-30 min. incubation of the primary Ab and BOND Refine (DS9800) or Bond Prime Polymer DAB Detection system (DS9284). Using these protocol settings, 29 of 37 (78%) laboratories produced a sufficient result. Applying VRPS, the proportion of

sufficient results was 82% (18/22) and 55% (12/22) were optimal (see Tables 1 and 3). *Seven laboratories used the RTU format off-label (e.g. deviant platforms).*

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), 10-30 min. incubation of the primary Ab and EnVision FLEX with or without Linker (GV800/GV823 + GV821) as detection system. Using these protocol settings, 51 of 51 (100%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was also 100% (22/22), 55% (12/22) optimal (see Tables 1 and 3). *Two laboratories used the RTU format off-label (deviant platform).*

mAb clone **DAK-CD10,** product no. **IR/IS786,** Dako/Agilent, Autostainer+/Autostainer Link 48:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 95-97°C), 20 min. incubation of the primary Ab and EnVision Flex+ (K8000/K8002 + K8021) as detection system. Using these protocol settings, 3 of 3 (100%) laboratories produced an optimal result. Applying VRPS, the proportion of sufficient results was 80% (4/5) and 40% (2/5) were optimal (see Tables 1 and 3). *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:
Many different protocols could be used for optimal results but showed to be less reproducible – see comment section below.
The most reproducible protocol settings with optimal results were typically based on HIER in CC1 (efficient heating time 48-64 min. at 98-100°C), 4-12 min. incubation of the primary Ab and OptiView with amplification (760-700/760-099) for 4+4 min as detection system. Using these protocol settings, 8 of 8 (100%) laboratories produced an optimal result. 14% (18/133) of the laboratories applied VRPS of which 17% (n=3) provided an optimal result (see Tables 1 and 3) and a pass rate of 39% was seen. *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**	100% (14/14)	79% (11/14)	93% (13/14)	71% (10/14)
Dako AS mAb **56C6 IR/IS648**	(2/3)	(0/3)	71% (5/7)	57% (4/7)
Leica Bond III/ Max/PRIME mAb **56C6 PA270/0131**	82% (18/22)	55% (12/22)	65% (13/20)	55% (11/20)
Dako Omnis mAb **DAK-CD10 GA786**	100% (22/22)	55% (12/22)	97% (30/31)	61% (19/31)
Dako AS mAb **DAK-CD10 IR786**	80% (4/5)	40% (2/5)	80% (8/10)	40% (4/10)
VMS GX/Ultra rmAb **SP67 790-4506**	39% (7/18)	17% (3/18)	62% (71/115)	26% (30/115)

* 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 65% (70/105) of the insufficient results. The remaining 35% of the insufficient results were mainly characterized by poor signal-to-noise ratio, excessive background, false positive staining reaction, 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 clone SP67 with a very sensitive protocol using OptiView with OptiView Amplification kit as detection system. The false positive staining result was characterized by an aberrant granular membranous and cytoplasmic staining reaction of most neoplastic cells in the non-GCB DLBCL – see Fig. 4b.

19% (77/403) of the participants used Abs as concentrated formats within laboratory developed (LD) assays for CD10 with 70% (54/77) producing a sufficient result, 60% (46/77) optimal. Within these, 96% (74/77) used the mAb clone 56C6, most commonly applied 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 was observed between the visualization systems used as well as related to the origin/vendor of the mAb clone 56C6 – see Table 1.
OptiView with or without amplification (760-700/760-099) was the most widely used visualization system within the participants who used the mAb clone 56C6 as a concentrate. Overall, 54% (40/74) of laboratories used this system of which 83% (33/40) obtained a sufficient result, 78% (31/40) optimal. Although the data is limited, similar to what was seen in the previous CD10 assessment run 66, 57% (4/7) of participants who did not pass using OptiView, used the mAb clone 56C6 concentrate produced by Cell Marque (110M-14/15/16, dilution factor between 1:20-1:50). When excluding the Cell Marque product, which itself had a pass rate of 14% (1/7) (see Table 1), the pass rate for protocols based on OptiView with or without amplification as the visualization system was 91% (32/35), 86% (30/35) optimal (Ab typically diluted in the range of 1:10-1:75).
Laboratories using Bond Refine (DS9800, Leica Biosystems) as visualization system obtained a pass rate of 67% (10/15), 47% (7/10) optimal (Ab typically diluted in the range of 1:40-1:50).
Laboratories using the Envision FLEX detection system with linker (K8000/K8002, GV800/GV823 + GV821, Dako/Agilent) had a pass rate of 100% (6/6), 67% (4/6) optimal (Ab typically diluted in the range of 1:10-1:50).
Generally, it was noted that sufficient HIER in an alkaline buffer together with an appropriate antibody dilution factor and a 3-step detection system were prerequisites for an adequate staining reaction. A shorter time in HIER and/or mAb required a lower dilution of the antibody concentrate in order to demonstrate CD10 in clinically relevant tissues such as distinct membranous staining reaction in over 30% of the neoplastic cells in the Diffuse Large B-Cell Lymphoma (DLBCL) of Germinal Centre B-cell (GCB) subtype. 4 participants used a citrate based acidic HIER buffer, none received a sufficient result.

Out of all the participants, 81% (326/403) used a ready-to-use (RTU) system to detect CD10. In this assessment and concordant to the previous run 66, 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% (115/128) and 61% (78/128) optimal, irrespective of the protocol applied.

The Leica Biosystems RTU system based on mAb clone 56C6 (PA0270/0131) was used by 12% (49/403) of all participants and resulted in an overall pass rate of 76% (37/49), 57% (28/49) optimal. A higher proportion of sufficient results was obtained using vendor recommended protocol settings (VRPS) compared to laboratory modified protocols (LMPS) on Leica staining platforms as the pass rates were 81% (17/21) and 65% (13/20), respectively (see Table 3). All of the insufficient results showed a generally too weak staining reaction, especially in the DLBCL of GCB-subtype, tissue core no. 4, where >30% of neoplastic cells were expected to show a distinct membranous staining reaction. In most cases no specific protocol settings inclusive different lot numbers could be pointed out that could explain the lower analytical sensitivity causing the unexpected inferior performance. However, internal data has indicated that the mAb clone 56C6 in working dilutions from the concentrated format or as an RTU from a different vendor can be prone to a shorter shelf-life and lower on-board stability, but if the same applies for the RTU system produced by Leica Biosystems is still to be elucidated. 7 participants used the antibody tailored for the Leica Bond stainers on the Ventana BenchMark Ultra with a pass rate of 86% (6/7), 71% (5/7) optimal.

The number of participants using the Dako/Agilent RTU systems based on the mAb clone 56C6 (GA648 and IR/IS648) decreased significantly as the company is terminating these products and replacing them with the new mAb clone DAK-CD10 based RTU systems (GA786 and IR786 for Omnis and Autostainer Link 48, respectively). 8% (32/403) of participants used the GA648 compared to 13% (50/393) as seen in the

previous assessment (run 66, 2022). A 100% (14/14) pass rate was achieved when using the product according to VRPS, 79% (11/14) of them optimal. The most common modification was a change in the incubation times of the antibody or HRP-conjugated polymer of the FLEX visualization system, however changes in these parameters did not impact the proportion of sufficient results. The only participant receiving an insufficient result used a low pH HIER buffer (GV805) instead of the standard Target Retrieval Solution, High pH which resulted in a false negative staining reaction. Four participants used the GA648 product on deviant platforms of which all 3 laboratories using the antibody on the Ventana Ultra platform produced an optimal result, while one laboratory using it on the Autostainer did not pass.

A 56% decrease was seen in the number of laboratories using the Dako/Agilent RTU system based on mAb clone 56C6 for Dako Autostainer (IR/IS648) as 16 participants used the product compared to 36 in the previous CD10 assessment run. 63% (10/16) of participants used it on the intended staining platform. No optimal results were seen when using the antibody per VRPS, while adding a linker resulted in a pass rate of 67% (4/6), all 4 results being optimal. Although all 6 laboratories used the same protocol settings, two of them showed a too weak or a false negative staining reaction, caused by either completely impaired morphology most likely connected to HIER in TRS High pH (3-in-1) or an uneven staining reaction, which might indicate issues with the levelling of the slide-racks on the Autostainer Link 48 stainer platform.
6 laboratories used the IR/IS648 product on deviant platforms (Ventana BenchMark XT/Ultra) or for manual staining, 100% (6/6) produced a sufficient result, 83% (5/6) optimal.

The most recently launched Dako/Agilent RTU systems based on the mAb clone DAK-CD10 for Dako Omnis (GA786) and for Dako Autostainer Link 48 (IR786) together were used by 19% (75/403) of participants and produced an overall pass rate of 93% (70/75). The mAb product developed for the Dako Omnis used according to VRPS achieved a pass rate of 100% (22/22), 55% (12/22) optimal. Similar to the previous CD10 assessment run, the most widely used protocol modification was adding a Mouse Linker, which was done by 25 participants and provided a pass rate of 96% (24/25), 60% (15/25) optimal. However, raising the analytical sensitivity of protocols for mAb clone DAK-CD10 with the addition of Mouse Linker increases the risk of unspecific background staining which might interfere with the interpretation as seen in the only unsuccessful result using GA786. Also, 78% (7/9) of successful results were downgraded to Good due to excessive background when a Linker was used. It is evident, that mAb clone DAK-CD10 has proven in the last two CD10 assessment runs to be suitable for the purpose of differentiating between DLBCL GCB and non-GCB subtype as well as for identifying clear cell renal cell carcinoma, but at present no data is available on the impact of diagnostic sensitivity and specificity in broader applications when the protocol is changed using Mouse Linker. 2 participants used the mAb clone DAK-CD10 developed for Dako Omnis on the Ventana BenchMark Ultra, both being optimal.

The mAb clone DAK-CD10 launched for Dako/Agilent Autostainer Link 48 (IR786) staining platform provided a pass rate of 80% and optimal rate of 40% using either VRPS or LMPS (see Table 3). Five participants used the IR786 product on deviant platforms (Leica BOND III, Ventana BenchMark Ultra) with the proportion of sufficient result being 80% (4/5), 60% (3/5) 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 the previous two (run 59, 2020 and run 66, 2022) and in the current assessment of CD10, the most widely used (133/403) and at the same time the least successful RTU system was the Ventana/Roche 790-4506 based on the rmAb clone SP67. The pass rate of the product has been fluctuating, being 59% in 2020, going down to 29% in 2022 and rising to 59% again in this assessment, indicating the technical difficulty laboratories are facing to obtain the reproducibility using the product. In line with the results from run 59 and 66, no specific parameters causing the low pass rate could be identified as similar protocol settings produced different results at different customer sites. As the purpose of the NordiQC assessment for CD10 has been the same for the past 3 runs, differentiating DLBCL GCB from non-GCB subtype, protocols and results from the three runs were pooled and analysed to see whether there are any common protocol settings that have shown to be more robust. 23 slides from 13 participants that were stained with Optiview together with Optiview Amplification kit, had HIER in high pH CC1 for 48-80 min. and antibody incubation between 4-12 min. (see Figs. 6a-6b) were assessed as sufficient in 87% (20/23) and optimal in 43% (10/23) of cases. Increasing either the time in HIER, the incubation of the mAb and/or OptiView Amplification kit reagents might easily result in a false positive staining in the neoplastic cells 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. In this assessment 54 participants used

OptiView with OptiView Amplification kit with a pass rate of 50% (27/54). 81% (22/27) of the insufficient results were caused by a false positive staining reaction or an overall granular precipitate interfering interpretation (see Figs. 1b-4b). The highest proportion of sufficient results for protocols based on OptiView without amplification was achieved together with 48-64 min. in HIER and antibody incubation of 32 min., which resulted in a pass rate of 78% (14/18 slides from 11 different laboratories), but only 14% (3/18) were optimal. Both a too weak and also false positive staining was seen in the 4 insufficient results, proving again the lack of robustness of the antibody.

The overall pass rate in the seventh assessment of CD10 was 73%, which is an increase from 64% obtained in the previous assessment in run 66, 2022. As seen in the latest NordiQC assessments, laboratories are switching from concentrated formats to RTU formats of Abs and in this run 81% used RTU formats compared to 77% in run 66, 2022.
The RTU systems based on mAb clone 56C6 (Leica Biosystems and Dako/Agilent) and the new mAb clone DAK-CD10 (Dako/Agilent) showed very similar high pass rates either with the VRPS (91% sufficient, 56% optimal) or LMPS (85% sufficient, 62% optimal) compared to the results from the previous assessment, proving the robustness of the products. As seen before, the Ventana/Roche RTU system based on rmAb clone SP67 showed a reduced reproducibility especially indicated by similar protocols giving different results and provided a low overall pass rate of 59% (78/133). 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 – 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.

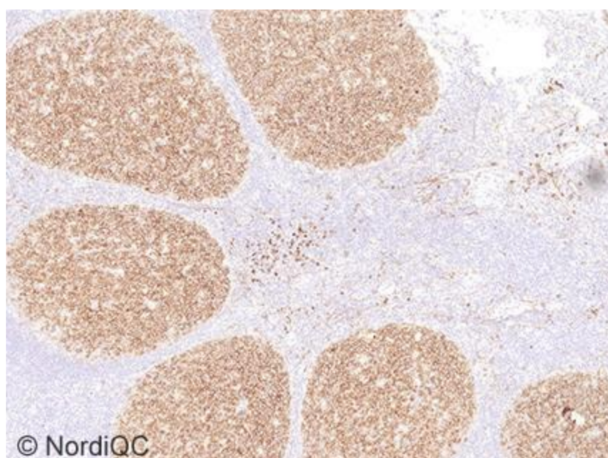


Fig. 1a
Optimal staining reaction for CD10 in tonsil using the mAb clone 56C6 (RTU, PA0270/0131, Leica Biosystems) per vendor recommended protocol settings (VRPS) on the Leica BOND III stainer platform seen by a low power magnification. All germinal centre B-cells and scattered neutrophil granulocytes 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.

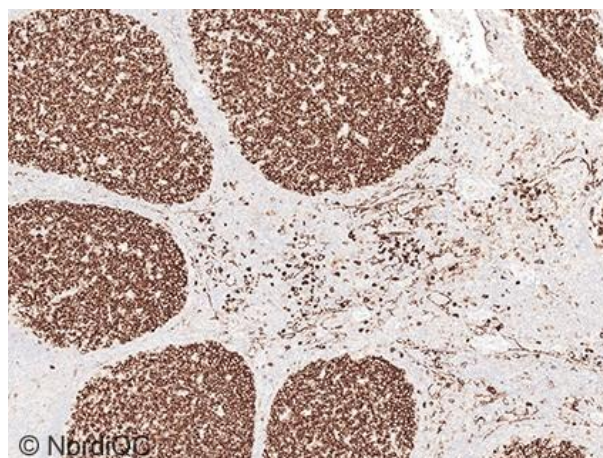


Fig. 1b
Insufficient staining reaction for CD10 in the tonsil using rmAb clone SP67 (RTU, 790-4506, Ventana/Roche) per VRPS and OptiView with OptiView Amplification Kit as detection system on the Ventana BenchMark Ultra. Germinal centre B-cells show a strong staining reaction, but also weak granular precipitates are seen covering the whole tissue, most likely caused by the tyramide based OptiView Amplification Kit. Both dispersed neutrophil granulocytes and scattered stromal cells become strongly positive. Same protocol used in Figs. 2b-4b – same field as 1a.

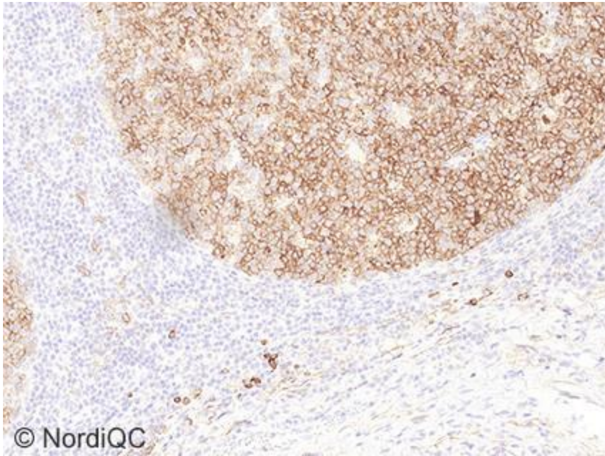


Fig. 2a
Optimal staining reaction for CD10 in tonsil using same protocol as in Fig. 1a. All germinal centre B-cells and scattered neutrophil granulocytes show a moderate to strong distinct membranous staining reaction. The mantle zone B-cells are negative. Scattered stromal cells display a weak membranous/cytoplasmic staining reaction.

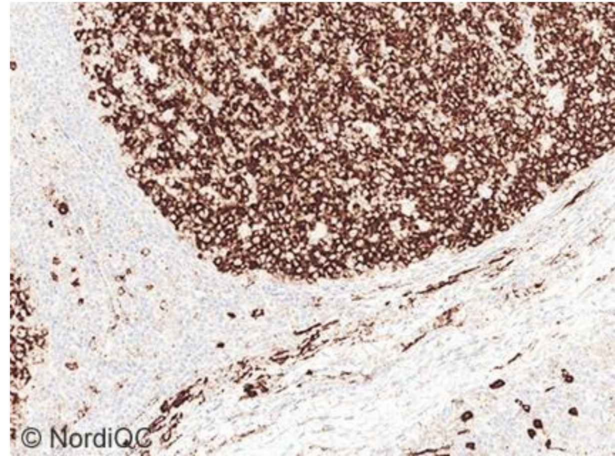


Fig. 2b
Insufficient staining reaction for CD10 in the tonsil using same protocol as in Fig. 1b. A strong staining reaction in the germinal centre B-cells is seen, however it is hard to discern a clear membranous staining reaction due to the signal being excessively amplified. Scattered stromal cells and neutrophil granulocytes display a strong staining reaction. Same field as 2a.

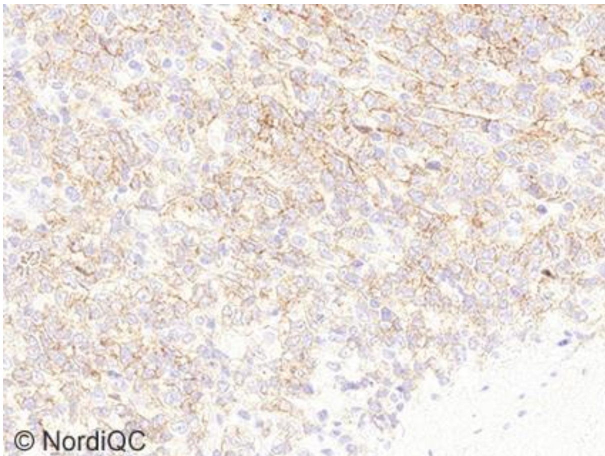


Fig. 3a
Optimal staining reaction for CD10 in the DLBCL (GCB subtype) using the same protocol as in Figs. 1a-2a. More than 30% of the neoplastic B-cells show a distinct membranous staining reaction.

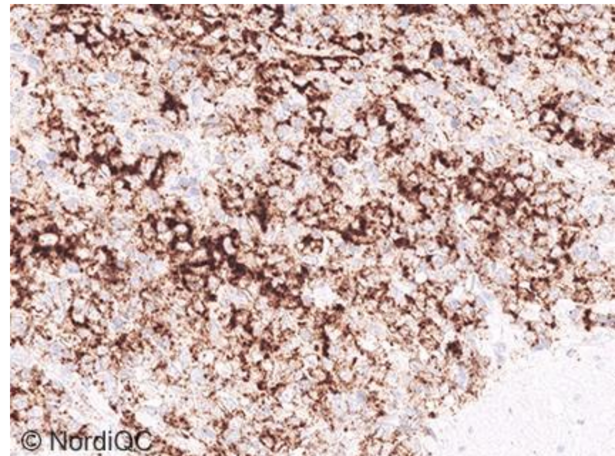


Fig. 3b
Insufficient staining reaction for CD10 in the DLBCL (GCB subtype) using same protocol as in Figs. 1b-2b. Same field as 3a. The majority of neoplastic B-cells show a weak to strong granular mostly membranous staining reaction. The amplified signal hides the morphology of the cells which interferes interpretation.

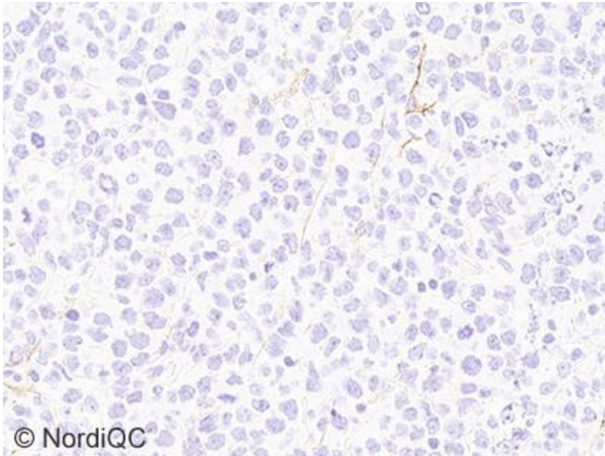


Fig. 4a
Optimal staining reaction for CD10 in the DLBCL (non-GCB subtype) using the same protocol as in Figs. 1a-3a. All neoplastic cells are negative while scattered stromal cells display a weak to moderate membranous/ cytoplasmic staining reaction.

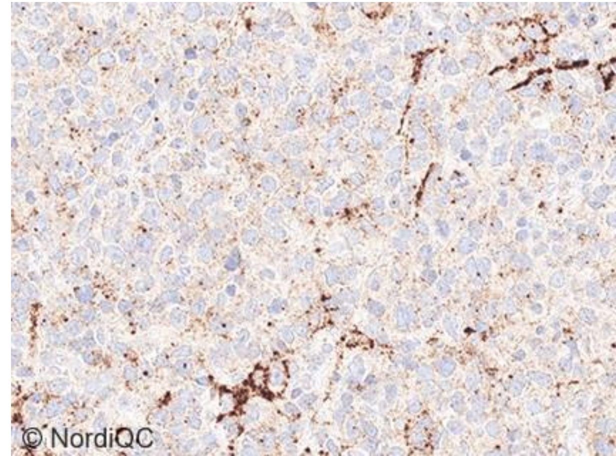


Fig. 4b
Insufficient staining reaction for CD10 in DLBCL (non-GCB subtype) using same protocol as in Figs. 1b-3b. Same field as 4a. Virtually all the neoplastic B-cells show a granular staining reaction with focal accentuation of the membranes resulting in a false positive staining. Scattered stromal cells display a strong granular membranous/cytoplasmic staining reaction.

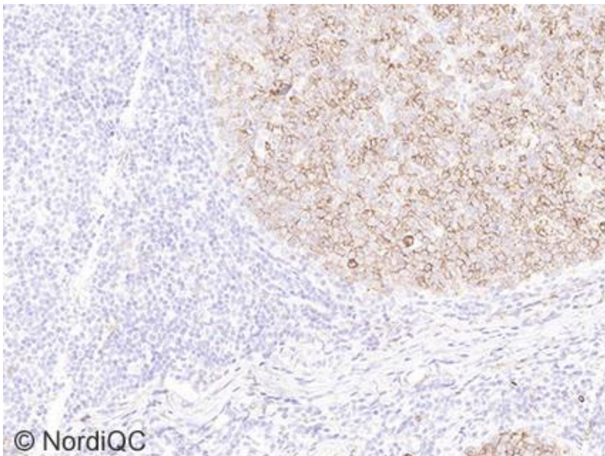


Fig. 5a
Insufficient staining reaction for CD10 in the tonsil using mAb clone 56C6 as a concentrate (1:20) from Cell Marque (30 min.), HIER in acidic BERS1 buffer (30 min.) and Bond™ Refine as detection system. A weak to moderate and less distinct staining reaction in a reduced proportion of germinal centre B-cells is seen. Many of the expected stromal cells and neutrophil granulocytes remain negative and only a weak staining reaction can be seen. Compare to same field in Fig. 2a.

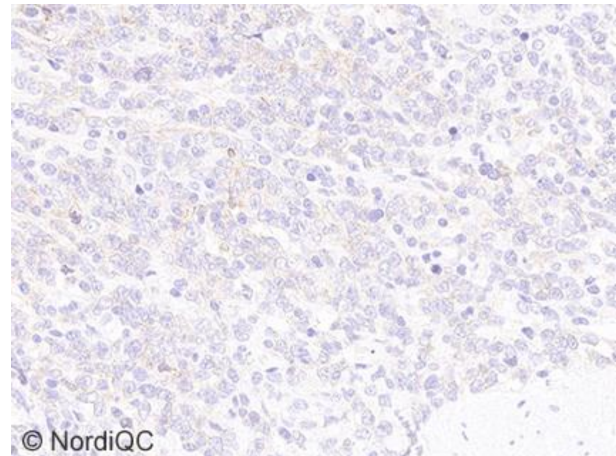


Fig. 5b
Insufficient staining reaction for CD10 in the DLBCL (GCB subtype) using the same protocol as in Fig. 5a. The majority of the neoplastic B-cells are false negative, only showing a faint cytoplasmic staining reaction, no distinct membranous accentuation can be identified compromising the diagnostic use in a clinical setting - compare with same field in Fig. 3a.

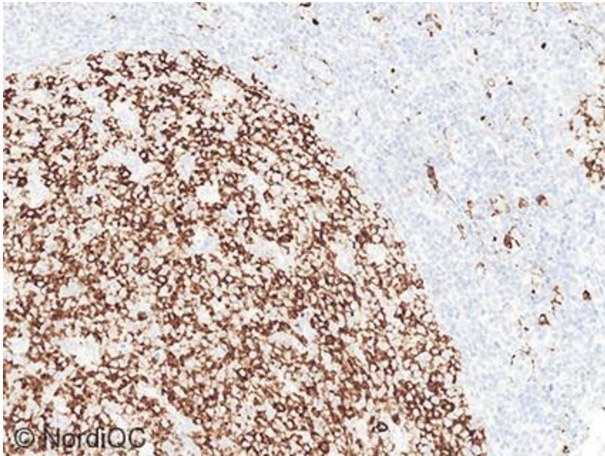


Fig. 6a
Optimal staining reaction for CD10 in the tonsil using the rmAb clone SP67 (RTU, 790-4506, Ventana/Roche), HIER in CC1 (64 min.), mAb incubation for 4 min. and OptiView with OptiView Amplification Kit (4+4 min.) as visualization system on the Ventana BenchMark Ultra stainer platform. The germinal centre B-cells and scattered neutrophil granulocytes show a distinct although focally partial membranous staining reaction.

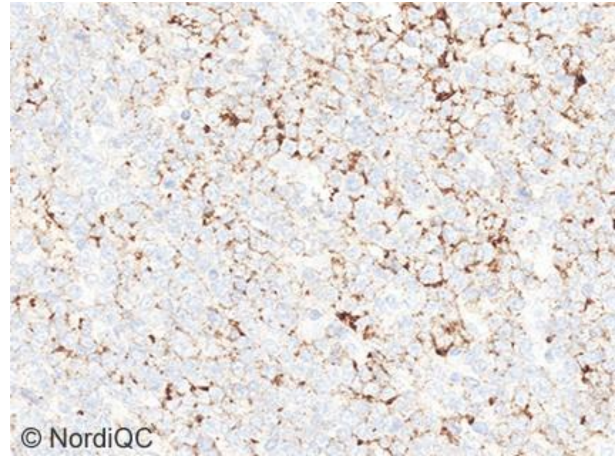


Fig. 6b
Optimal staining reaction for CD10 in the DLBCL (GCB subtype) using the same protocol as in Fig. 6a. More than 30% of the neoplastic B-cells show a distinct membranous staining reaction.

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