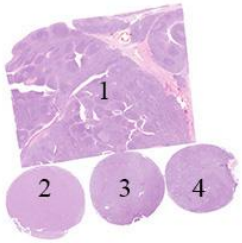
Assessment Run 69 2023
CD5

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD5, typically used in the diagnostic work-up of hematological neoplasms to identify T-cells as well as T-cell lymphomas and selected B-cell neoplasms. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD5 (see below).

Material
The slide to be stained for CD5 comprised:



1. Tonsil, 2. Diffuse large B-cell lymphoma (DLBCL), 3. Mantle cell lymphoma (MCL), 4. B-cell chronic lymphatic leukemia (B-CLL).

All tissues were fixed in 10% neutral buffered formalin.
Criteria for assessing a CD5 staining as optimal included:

- A strong and distinct, predominantly membranous staining reaction of virtually all T-cells in both T-zones and in germinal centers in the tonsil.
- An at least weak to moderate, distinct membranous staining reaction of the majority of B-cells in the mantle zones of the tonsil.
- A moderate to strong, distinct membranous staining reaction of all neoplastic cells in the B-CLL.
- A weak to moderate, but distinctly membranous staining reaction of all the neoplastic cells in the MCL.
- A strong, distinct membranous staining reaction of all T-cells intermingling with the neoplastic cells of the DLBCL, MCL and B-CLL.
- No staining reaction of the germinal center B-cells and neoplastic cells in the DLBCL.

Participation

Number of laboratories registered for CD5, run 69	416
Number of laboratories returning slides	379 (91%)

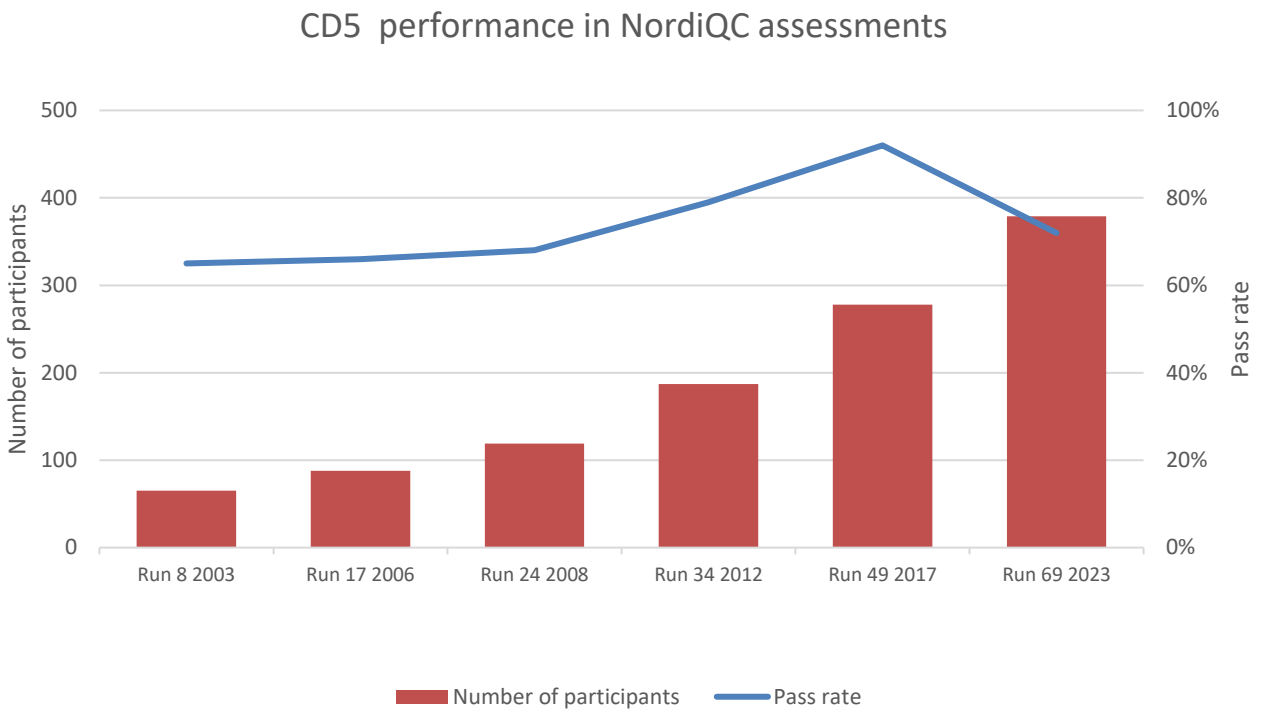
Results
At the date of assessment, 91% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

379 laboratories participated in this assessment, 272 (72%) 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 reactions were:
- Inefficient Heat Induced Epitope Retrieval (HIER) e.g., too short HIER time and/or use of citric based HIER buffer
- Too diluted and too short incubation time in primary antibody
- Less sensitive detection systems
- Less successful mitigation of Dako Autostainer Link 48 RTU system IR/IS082 to Dako Omnis staining platform
- Less successful primary antibodies

Performance history
This was the sixth NordiQC assessment of CD5. The pass rate has decreased compared to the results obtained in run 49, 2023 (see Graph 1).

Graph 1. **Proportion of sufficient results for CD5 in the six NordiQC runs performed**



Conclusion
The mAb clones **4C7, C6A10**, **DA029** and rmAb clones **SP19, IHC738, QR111, 164G9B4, BP6090, BY127** could all be used to obtain an optimal staining reaction for CD5. In this assessment, all insufficient results were characterized by a too weak and indistinct or false negative staining reaction. The rmAb clone SP19 proved to be the most robust antibody for the detection of CD5 achieving a pass rate of 95% (177/187) with 81% optimal across all formats and products based on that clone. 50% (53/107) of all insufficient staining reactions were caused by a less successful performance or the mAb clone 4C7 based Dako/Agilent RTU system IR/IS082 for Autostainer Link 48. 68% (36/53) of these were the result of an unsuccessful mitigation of the RTU system to the Dako Omnis staining platform. Main prerequisites for sufficient and optimal results were the use of HIER based on an alkaline buffer, careful calibration of the primary antibody and the use of sensitive 3-step detection systems. In this assessment, the RTU systems from Leica Biosystems and Ventana/Roche were most successful providing a pass rate of 100% and 91%, respectively when applied by vendor recommended protocol settings.
Tonsil is recommended as positive and negative tissue control for CD5. Virtually all T-cells must show a strong membranous staining reaction, while an at least weak to moderate and distinct membranous staining reaction must be seen in dispersed mantle zone B-cells in secondary follicles. No staining reaction must be seen in the germinal center B-cells.

Table 1. **Antibodies and assessment marks for CD5, run 69**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4C7**	44	Leica Biosystems	21	11	24	3	54%	36%
	11	Dako/Agilent						
	1	Biocare Medical						
	1	Cell Marque						
	1	DCS						
	1	Epredia						
mAb clone **CD5/54/F6**	1	Santa Cruz	-	-	-	1	-	-
rmAb clone **EP2952**	1	Epitomics	-	-	1	-	-	-
rmAb clone **EP77**	1	Abcam	-	-	1	1	-	-
	1	PathnSitu						
rmAb clone **IHC738**	1	GenomeMe	1	-	-	-	-	-
rmAb clone **QR111**	1	Quartett	1	-	-	-	-	-
rmAb clone **RBT-CD5**	1	Bio SB	-	-	-	1	-	-
rmAb clone **SP19**	1	Abcam	7	2	2	-	82%	64%
	6	Cell Marque						
	1	Diagnostic BioSystems						
	1	Epredia						
	2	Zytomed Systems						
rmAb clone **ZR228**	2	Zeta Corporation	-	-	2	-	-	-
Conc total	79		30	13	30	6	54%	38%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **4C7 IR/IS082 (VRPS)[3]**	11	Dako/Agilent	2	2	6	1	36%	18%
mAb clone **4C7 IR/IS082 (LMPS)**[4]	69	Dako/Agilent	12	11	40	6	33%	17%
mAb clone **4C7 PA0168 (VRPS)**[3]	13	Leica Biosystems	5	8	-	-	100%	38%
mAb clone **4C7 PA0168 (LMPS)**[4]	16	Leica Biosystems	2	9	3	2	69%	13%
mAb clone **4C7 AM430-5/10**	1	BioGenex	1	-	-	-	-	-
mAb clone **4C7 205M-17/18**	1	Cell Marque	-	-	1	-	-	-
mAb clone **4C7 PDM095**	1	Diagnostic BioSystems	1	-	-	-	-	-
mAb clone **C6A10 CCM-0354**	1	Celnovte	1	-	-	-	-	-
mAb clone **DA029 RMF1A059**	1	Shenzhen Dartmon Biotechnology	1	-	-	-	-	-
rmAb clone **SP19 790-4451 (VRPS)**[3]	22	Ventana/Roche	16	4	2	-	91%	73%
rmAb clone **SP19 790-4451 (LMPS)**[4]	152	Ventana/Roche	127	20	3	2	97%	84%
rmAb clone **SP19 205R-17/18**	1	Cell Marque	1	-		-	-	-
rmAb clone **SP19 RMPD011**	1	Diagnostic BioSystems	-	-	-	1	-	-
rmAb clone **EP77 MAD-000602QD**	3	Master Diagnostica	-	-	2	1	-	-
rmAb clone **164G9B4 PA221**	1	Abcarta	1	-	-	-	-	-
rmAb clone **BP6090 I10652E-05**	1	Biolynx	1	-	-	-	-	-
rmAb clone **BY127 BFM-0445**	1	Bioin Biotechnology	1	-	-	-	-	-
rmAb clone **GR020 8249-C010**	1	Sakura Finetek	-	1	-	-	-	-
rmAb clone **IHC738**	1	GenomeMe	-	1	-	-	-	-

rmAb clone **MX052 MAB-0827**	1	Fuzhou Maixin Biotech	1	-	-	-	-	-
Ab clone **DGR008**	1	Shanghai DG Diagnology Tec	-	-	-	1	-	-
RTU total	300		173	56	57	13	76%	58%
Total	379		203	69	87	19		
Proportion			54%	18%	23%	5%	72%	

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 CD5, Run 69
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb **4C7**: Protocols with optimal results were all based on HIER using alkaline buffer as Cell Conditioning 1 (CC1, Ventana/Roche) (9/18)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (1/3), Target Retrieval Solution (TRS) High pH (Dako/Agilent) (5/14) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (6/17) as retrieval buffer. The mAb was typically diluted in the range of 1:40 – 1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings, 17 of 35 (71%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

rmAb **SP19**: Protocols with optimal results were based on HIER using alkaline buffer as CC1 (Ventana/Roche) (2/3), TRS pH 9 (3-in-1) (Dako/Agilent) (1/1), TRS High pH (Dako/Agilent) (3/3) or Dewax and HIER Buffer H (Epredia) (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:20 – 1:60 depending on the total sensitivity of the protocol employed. Using these protocol settings, 9 of 9 (100%) laboratories produced a sufficient staining.

Table 2. **Proportion of optimal results for CD5 for the two most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark Ultra/GX**		**Leica Biosystems Bond III , Max, PRIME**	
	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 **4C7**	1/3	-	5/14 (36%)	-	9/18 (50%)	-	6/17 (35%)	0/5 (0%)
rmAb clone **SP19**	1/1	-	4/4	-	2/3	0/1	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 **4C7,** product no. **IR/IS082,** Dako/Agilent, Autostainer Link 48:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 97-98°C), 20 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002+K8021) as detection system. Using these protocol settings, 4 of 5 (80%) laboratories produced a sufficient result.

mAb clone **4C7,** product no. **PA0168,** Leica Biosystems, Bond III:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20-30 min. at 95-100°C), 15-20 min. incubation of the primary Ab and BOND Refine (DS9800) as detection system. Using these protocol settings, 19 of 19 (100%) laboratories produced a sufficient result.

rmAb clone **SP19,** product no. **790-4451,** Ventana/Roche, Benchmark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-64 min.) and 8-64 min. incubation of the primary Ab. UltraView (760-500) with amplification (760-080) or OptiView (760-700) were used as detection systems. Using these protocol settings, 95 of 96 (99%) laboratories produced a sufficient staining result.

Table 3. **Proportion of sufficient and optimal results for CD5 for the most commonly used RTU IHC systems**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 mAb 4C7 **IR/IS082**	36% (4/11)	18% (2/11)	53% (8/15)	40% (6/15)
Leica BOND III mAb 4C7 **PA0168**	100% (13/13)	38% (5/13)	67% (10/15)	7% (1/15)
VMS XT/Ultra/Ultra Plus rmAb SP19 **790-4451**	91% (20/22)	73% (16/22)	97% (144/149)	83% (124/149)

* 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 are integrated.

Comments

In this sixth NordiQC assessment of CD5, the prevalent feature of an insufficient result was a generally too weak staining reaction of cells expected to be demonstrated and/or a false negative staining result. This pattern was seen in 100% of insufficient results (n=107). The majority of laboratories were able to detect CD5 in high-level antigen expressing cells as T-cells (all specimens) and neoplastic cells of the B-CLL, whereas a distinct membranous demonstration of CD5 in the majority of mantle zone B-cells in the tonsils and the neoplastic B-cells of the MCL was significantly more challenging and required a carefully calibrated protocol.

The rmAb clone SP19 and mAb clone 4C7 were the most widely used antibodies for the demonstration of CD5. Used as a concentrate in a LD assay, mAb clone 4C7 gave an overall pass rate of 54% (32/59), 36% (21/59) optimal. As shown in Table 2, optimal results could be obtained on all main semi- and fully automated platforms. Efficient HIER in an alkaline buffer, careful calibration of the primary Ab and the use of a sensitive detection system were the most central parameters for optimal results. More specifically, all (n=7) protocols based on HIER in BERS2 and antibody dilution factor between 1:40-1:100 resulted in a sufficient staining when preformed on the Leica Bond III staining platform. On the Omnis staining platform from Dako/Agilent, all protocols assessed as optimal were based on HIER in TRS High pH for 30 min., antibody dilution factor between 1:40-1:100 and the use of a 3-step FLEX+ detection system. Using these protocol settings, 6 of 7 (86%) laboratories produced a sufficient staining result. Within participants using the mAb clone 4C7 as a concentrate on the Ventana Benchmark Ultra staining platform, the highest proportion of sufficient (82%, 9/11) and optimal (73%, 8/11) results were obtained with protocols based on HIER in an alkaline CC1 buffer and the use of OptiView as detection system, regardless of antibody dilution factor applied (ranged between 1:50-1:400).

The rmAb clone SP19 used as concentrated format within a LD assay provided a relatively high pass rate of 82% (9/11). Optimal results could be obtained on most of the main IHC systems from Ventana/Roche (Benchmark Ultra) and Dako/Agilent (Autostainer Link 48, Omnis) together with HIER in an alkaline buffer and a relatively high antibody dilution factor between 1:25-1:60. All but two Omnis users also applied a 3-step detection system, either OptiView for Ventana Benchmark Ultra or FLEX+ for Dako Autostainer Link 48 or Omnis. The only slide stained on the Leica Bond III staining platform was scored as insufficient due to a too weak staining reaction most likely caused by a too low titre of the antibody (1:75).

Ready-To-Use (RTU) antibodies were used by 79% (300/379) of laboratories.
The Ventana/Roche RTU system based on rmAb clone SP19 (790-4451) was the most widely used RTU system applied by 174 laboratories. A very high overall pass rate of 96% (167/174) was seen, 82% (143/174) optimal. Although vendor recommended protocol settings (VRPS) could be used to obtain a sufficient staining result, the vast majority of participants used laboratory modified protocol settings (LMPS). The latter mostly focused on raising the sensitivity of the staining protocol with either changing the detection system from vendor recommended UltraView to OptiView and/or modifying incubation times, resulting in a slightly higher pass rate of 97% (147/152), 84% (127/152) optimal.

The Dako/Agilent RTU system IR/IS082 based on mAb clone 4C7 for Autostainer Link 48 was used by 21% (80/379) of the participants and provided a low pass rate of 34% (27/80), 18% (14/80) optimal. That was largely caused by an unsuccessful off-label use of the antibody on the fully-automated Dako Omnis staining platform as no vendor validated CD5 RTU systems are currently available for it. In total, 51 participants used the IR/IS082 antibody on Dako Omnis resulting in a pass rate of 25% (13/15), 8% (4/51) optimal. In this assessment the IR/IS082 RTU system also provided a generally low pass rate of 36% (4/11) when used according to VRPS on the Autostainer Link 48, 18% (5/11) optimal, contrary to

the high pass rate of 95% as seen in the previous NordiQC CD5 assessment (run 49, 2017). Based on the data submitted by the participants, no inferior LOT numbers could be identified which could explain the drop in pass rate amongst laboratories following VRPS. All insufficient results obtained with IR/IS082 were characterized by a too weak and indistinct membranous staining reaction or a false negative result especially in the MCL (see Fig. 2b) and mantle zone B-cells in the tonsil (see Fig. 1b). Using a 3-layer detection system FLEX+ on both of the Dako/Agilent staining platforms significantly raised the pass rate to 81% (17/21), 43% (9/21) optimal, regardless of HIER or antibody incubation times. It must be emphasized that for laboratories wishing to use an off-label RTU format, focus on intended use requires thorough technical calibration and analytical/diagnostic validation.

For the Leica Biosystems RTU system PA0168 (BOND III/MAX), an overall pass rate of 83% (24/29) was obtained and 24% (7/29) were optimal. All participants following VRPS based on HIER in BERS2 for 20 min. and antibody incubation for 15 min. produced a sufficient staining result, 38% (5/13) were optimal (see Table 3). Among laboratories using LMPS, a reduction in HIER time or change of vendor recommended HIER buffer were the main reasons for insufficient staining results.

This was the sixth NordiQC assessment of CD5 and an overall pass rate of 72% was obtained, which is a significant decrease from run 49 in 2017 (92%, Graph 1). Half (53/107) of the unsuccessful results were attained with the use of Dako/Agilent RTU system IR/IS082 validated for Dako Autostainer Link 48, especially when it was used off-label on the fully-automated Dako Omnis staining platform resulting in a too weak and imprecise labeling of cells expected to be positive or a completely false negative staining reaction. The rmAb clone SP19 proved to be the most robust clone for the demonstration of CD5 achieving a pass rate of 95% (177/187) and a high proportion of optimal results (81%, 151/187) across all platforms, formats and products based on that clone. In general, efficient HIER in alkaline buffer, careful calibration of the primary Ab and the use of a sensitive 3-layer detection system were prerequisites for optimal results.

Controls

Tonsil is recommended as a positive and negative tissue control, in which dispersed B-cells in the mantle zone of the secondary follicles must display a weak to moderate and distinct membranous staining reaction. The proportion of CD5 positive B-cells in the mantle zone can vary from tonsil to tonsil, but the central aspect is to demonstrate distinct and sharp membranes of the individual cells. If these cells were negative or only faintly demonstrated, the proportion of positive neoplastic cells especially in the mantle cell lymphoma were reduced. Virtually all T-cells, both in the interfollicular T-zones and within germinal centres, must show a strong and distinct predominantly membranous staining reaction. No staining reaction must be seen in the germinal centre B-cells.

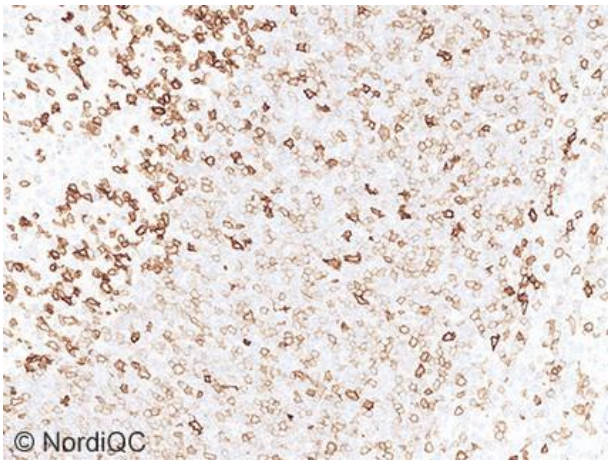


Fig. 1a
Optimal staining result for CD5 of the tonsil, tissue core no 1, using the rmAb clone SP19 (RTU, 790-4451, Ventana Roche) per vendor recommended protocol settings (VRPS) on the Ventana Benchmark Ultra stainer platform with HIER for 64 min. in an alkaline buffer (CC1), antibody incubation for 16 min. and UltraView as detection system - same protocol used in Figs. 2a - 4a. Dispersed B-cells in the mantle zone show a weak to moderate but distinct membranous staining reaction while the T-cells within the germinal centre show a strong distinct membranous staining reaction.

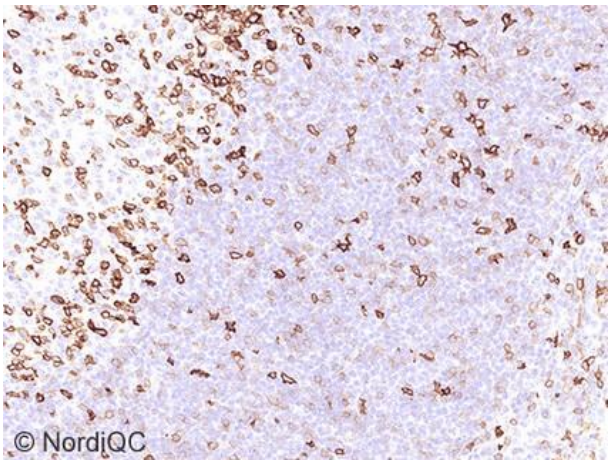


Fig. 1b
Insufficient staining result for CD5 of the tonsil, tissue core no 1, using the Dako/Agilent RTU system IR/IS082 based on mAb clone 4C7 off-label on Dako Omnis with HIER in TRS High pH for 30 min., antibody incubation for 20 min. and a 2-layer detection system FLEX - same protocol used in Figs. 2b – 4b. The intensity of the staining reaction in the mantle zone B-cells is significantly reduced with almost no B-cells showing a distinct membranous staining reaction - compare with Fig. 1a (same field). Dispersed T-cells still exhibit a strong membranous staining reaction.

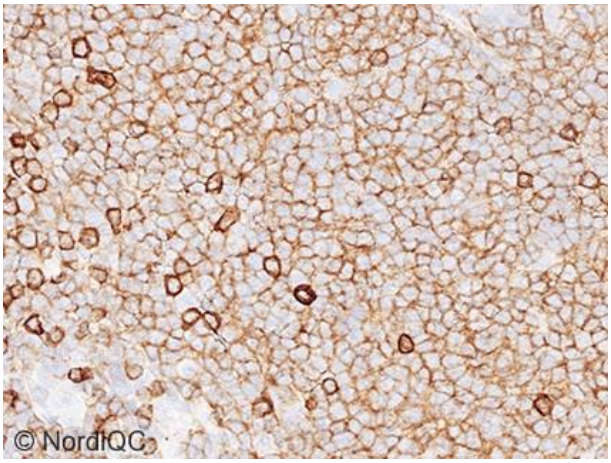


Fig. 2a
Optimal staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate distinct membranous staining reaction. T-cells intermingling with the neoplastic cells show a strong membranous staining reaction.

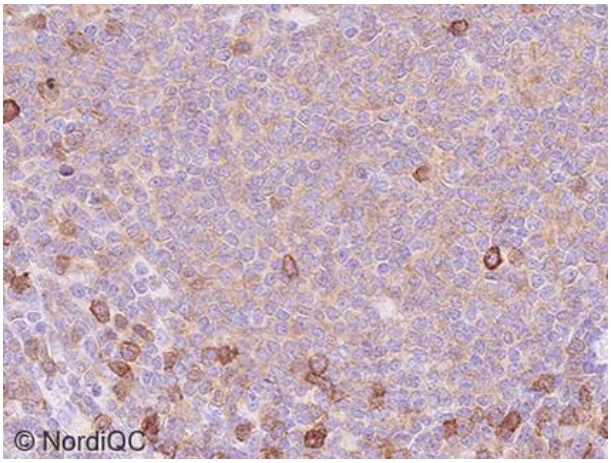


Fig. 2b
Insufficient staining result for CD5 of the MCL, tissue core no 3, using the same protocol as in Fig. 1b. The intensity of the neoplastic cells is significantly reduced and displays an indistinct staining reaction pattern - compare with Fig. 2a (same field).
T-cells intermingling with the neoplastic cells retain a strong membranous staining reaction.

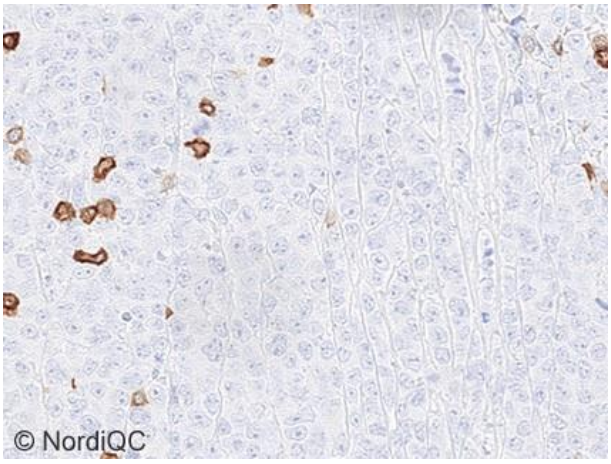


Fig. 3a
Optimal CD5 staining result of the DLBCL, tissue core no 2, using same protocol as in Figs. 1a and 2a. All the neoplastic B-cells are negative as expected. T-cells are distinctively demonstrated intermingling between the neoplastic B-cells.

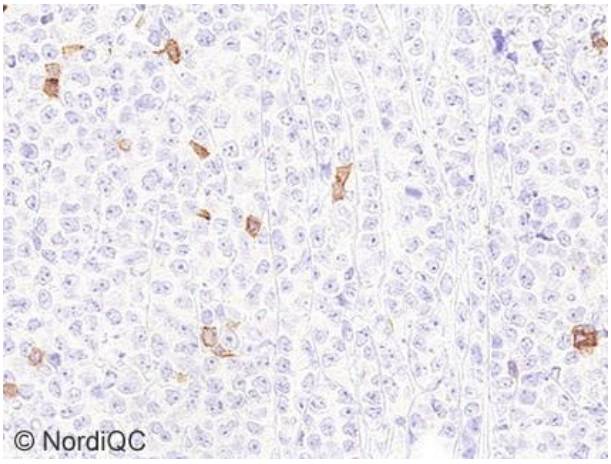


Fig. 3b
Staining for CD5 of the DLBCL, tissue core no 2, using the same protocol as in Figs. 1b and 2b. T-cells are clearly demonstrated despite being less intense compared to the result shown in Fig. 3a. The neoplastic B-cells are negative as expected.

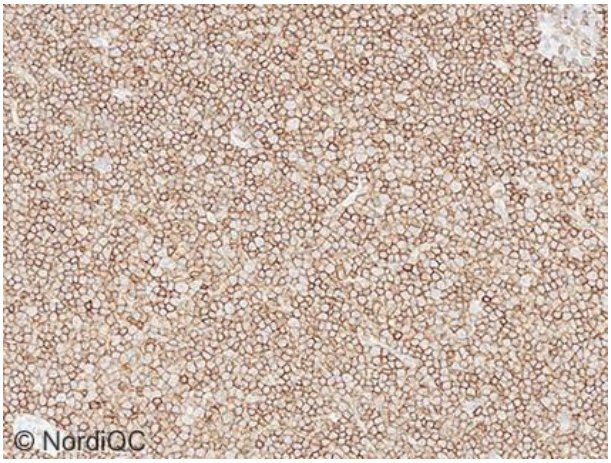


Fig. 4a
Optimal CD5 staining result of the B-CLL, tissue core no 4, using same protocol as in Figs. 1a - 3a. All the neoplastic cells show a moderate to strong and distinct membranous staining reaction.

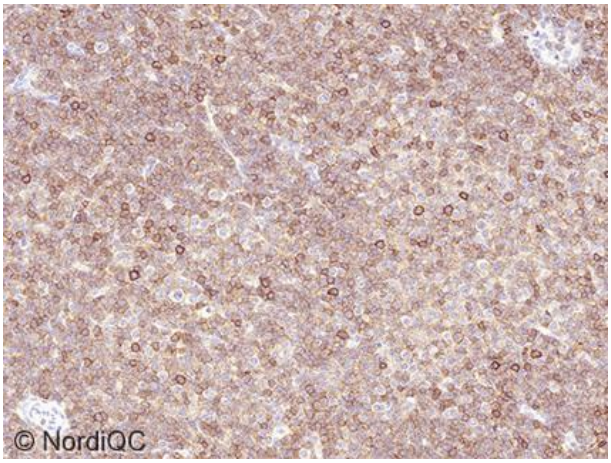


Fig. 4b
Staining result for CD5 of the B-CLL, tissue core no 4, using the same insufficient protocol as in Figs. 1b - 3b. The intensity of the neoplastic cells is slightly reduced compared to the level expected and the membranous staining reaction pattern is more indistinct - compare with Fig. 4a (same field).

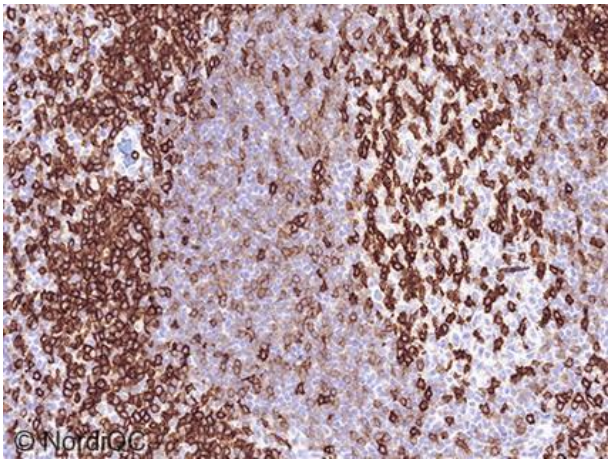


Fig. 5a
Optimal CD5 staining result of tonsil using the mAb clone 4C7 as a concentrate (dilution factor 1:50) on Dako Omnis with HIER in TRS High pH for 30 min., antibody incubation for 30 min. and a 3-layer detection system FLEX+, same protocol used in Fig. 5b. The T-cells in the interfollicular T-zone and within the germinal centre show a strong distinct membranous staining reaction. Of critical importance, dispersed B-cells in the mantle zone show a weak to moderate distinct membranous staining reaction.

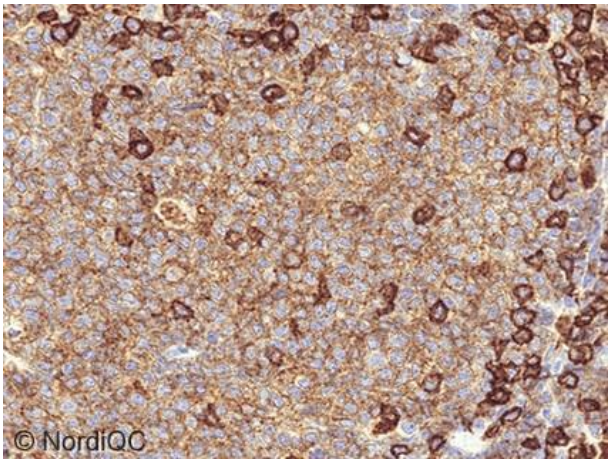


Fig. 5b
Optimal CD5 staining result of the MCL, tissue core no 3, using the same protocol as in Fig. 5a. Virtually all the neoplastic cells show a distinct, moderate membranous staining reaction while T-cells intermingling with the neoplastic cells show a strong membranous staining reaction.

BT/LE/SN 28.11.2023