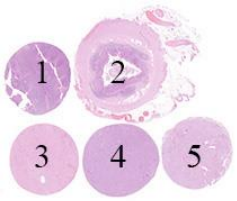
Assessment Run 67 2023

CD4

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD4, identifying and classifying T-cell lymphomas in the diagnostic work-up of hematological neoplasms. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD4 (see below).

Material
The slide to be stained for CD4 comprised:

1. Tonsil, 2. Appendix, 3. Liver, 4. Diffuse large B-cell lymphoma (DLBCL), 5. Angioimmunoblastic T-cell lymphoma (AITL).



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD4 staining as optimal included:

- A strong, distinct membranous staining reaction of virtually all helper/inducer T-cells in the T-zones and within the germinal centres of the tonsil.
- A moderate to strong, distinct membranous staining reaction of intraepithelial T-cells in the appendix.
- An at least weak, distinct predominantly membranous staining reaction of macrophages in germinal centres of the tonsil, in lamina propria of the appendiceal mucosa and Kupffer cells in the liver.
- An at least moderate staining reaction of virtually all endothelial cells in the liver sinusoids.
- An at least moderate, distinct membranous staining reaction of all neoplastic cells in the T-cell lymphoma.
- No staining reaction of other cells e.g., B-cells (all cores), squamous epithelial cells of the tonsil and columnar epithelial cells of the appendix. The neoplastic B-cells of the DLBCL should also be negative, but subpopulations of macrophages and normal T-helper/inducer cells should be distinctively demonstrated intermingling between the negative neoplastic B-cells.

Participation

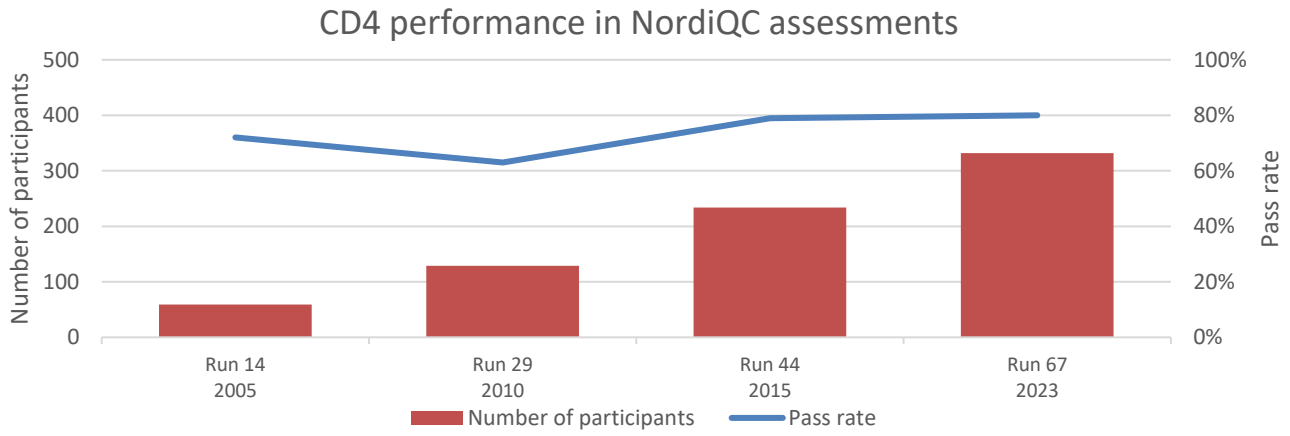
Number of laboratories registered for CD4, run 67	371
Number of laboratories returning slides	332 (89%)

Results
332 laboratories participated in this assessment. 265 (80%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3). 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.

The most frequent causes of insufficient staining reactions were:
- Inefficient HIER (too short heating time)
- Less successful performance of mAb clone 4B12 on the Omnis and Leica BOND platforms
- Unexplained technical issues

Performance history
This was the fourth NordiQC assessment of CD4. The pass rate was similar to the results obtained in the previous run 44, 2015 (see Graph 1).

Graph 1. **Proportion of sufficient results for CD4 in the four NordiQC runs performed**



Conclusion
The mAb clone **4B12** and the rmAb clones **SP35, EP204, IHC535, C9E15, 458G4A1** could all be used to obtain an optimal staining result for CD4. Irrespective of the clone applied, efficient HIER, use of appropriate primary Ab tailored to the choice of IHC system and careful calibration of the primary antibody were the most important prerequisites for an optimal performance. Using vendor recommended protocol settings, the Ready-To-Use (RTU) system 790-4423 (Ventana/Roche) based on the rmAb clone SP35 provided superior results and the highest proportion of optimal results among all the RTU systems (from the main vendors) but also in relation to laboratory developed (LD) tests. For the mAb clone 4B12, both as concentrates and RTU formats, the performance was highly influenced by the chosen platform e.g., for the Omnis (Dako/Agilent), all protocols (33/33) were assessed as insufficient.
Tonsil is recommended as positive and negative tissue controls: All helper/inducer T-cells must show a distinct and strong membranous staining reaction, while germinal centre macrophages must at least display a weak but distinct staining reaction. No staining reaction should be seen in B-cells and squamous epithelial cells of the tonsil.

Table 1. **Antibodies and assessment marks for CD4, run 67**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **4B12**	14 10 1 1	Leica Biosystems Dako/Agilent Biocare medical Epredia	2	12	5	7	54%	8%
mAb clone **1F6**	3	Leica Biosystems	0	1	2	0	-	-
rmAb clone **SP35**	32 2 1 1	Cell Marque Spring Biosciences Abcam SanBio	21	12	2	1	92%	58%
rmAb clone **EP204**	3	Epitomics	2	0	1	0	-	-
rmAb clone **IHC535**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **ZR110**	1	Zeta Corporation	0	0	1	0	-	-
rmAb clone **QR032**	1	Quartett	0	0	1	0	-	-
Conc total	71		26	25	12	8	72%	37%
Ready-To-Use antibodies								
mAb clone **4B12 PA0427**[3]	13	Leica Biosystems	1	9	3	0	77%	8%
mAb clone **4B12 PA0427**[4]	9	Leica Biosystems	2	2	3	2	44%	22%
mAb clone **4B12 IR649**[3]	14	Dako/Agilent	8	5	1	0	93%	57%
mAb clone **4B12 IR649**[4]	48	Dako/Agilent	11	7	15	15	38%	23%
rmAb clone **SP35 790-4423**[3]	31	Ventana/Roche	30	1	0	0	100%	98%
rmAb clone **SP35 790-4423**[4]	116	Ventana/Roche	105	8	2	1	97%	91%
rmAb clone **SP35 104R-17/18**	10	Cell Marque	6	3	1	0	90%	60%
rmAb clone **SP35 RMA-0620**	1	Fuzhou Maixin	1	0	0	0	-	-
rmAb clone **SP35 SC0135**	3	Spring Bioscience	2	0	1	0	-	-
rmAb clone **SP35 BRB042**	2	Zytomed systems	0	1	0	1	-	-
rmAb clone **EP204 MAD-000600QD**	4	Master Diagnostica	3	1	0	0	-	-
rmAb clone **EP204 8226-C010**	1	Sakura Finetek	0	1	0	0	-	-
rmAb clone **EP204 AN722-5M**	1	BioGenex	0	1	0	0	-	-
rmAb clone **EP204 PR013**	1	Pathn Situ	1	0	0	0	-	-
rmAb clone **EP204 Unknown**	1	Epitomics	0	0	0	1	-	-
rmAb clone **IHC535 IHC535-7**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **C9E15 CCR-0343**	1	Celnovte	1	0	0	0	-	-
rmAb clone **458G4A1 PA285**	1	Abcarta	1	0	0	0	-	-
Unknown	3		2	0	1	0	-	-
RTU total	261		175	39	27	20	82%	67%
Total	332		201	64	39	28	-	
Proportion			61%	19%	12%	8%	80%	

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 CD4, Run 67
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **4B12**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 6 (3-in-1) (Dako/Agilent) (1/1)* or Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (1/15) as retrieval buffer. The mAb was diluted in the range of 1:100-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings, 5 of 8 (63%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **SP35**: Protocols with optimal results were based on HIER using Cell Conditioning 1 (CC1; Ventana/Roche) (8/14), TRS pH 9 (3-in-1) (Dako/Agilent) (12/19) and BERS2 (Leica Biosystems) (1/2) as retrieval buffer. The rmAb was typically diluted in the range of 1:10-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 26 of 28 (93%) laboratories produced a sufficient staining result.

rmAb clone **EP204**: Protocols with optimal results were based on HIER buffer H (Epredia) (1/1) or Tris-EDTA / EGTA pH 9 (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:25-1:150 and EnVision Flex+ (Dako/Agilent) or GTVision (Gene Tech) were used as detection systems.

rmAb clone **IHC535**: One protocol with an optimal result was based on HIER using BERS2 (Leica Biosystems) as retrieval buffer. The rmAb was diluted 1:200 and Bond Refine was used as detection system.

Table 2. **Proportion of optimal results for CD4 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**		**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 **4B12**	0/1**	1/1	0/2	-	-	-	1/7 (14%)	-
rmAb clone **SP35**	0/1	-	11/14 (79%)	-	8/11 (73%)	-	1/2	-

* 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 **4B12**, product no. **PA0427**, Leica Biosystems, Bond-III:
Protocols with optimal results were based on HIER using BERS2 or Bond Epitope Retrieval Solution 1 (BERS1) (efficient heating time 20-30 min. at 100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 11 of 15 (73%) laboratories produced a sufficient staining result.

mAb clone **4B12**, product no. **IR649**, Dako/Agilent, Autostainer:
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), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002+K8022/K8021) as detection systems. Using these protocol settings, 14 of 14 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP35**, product no. **790-4423**, Ventana/Roche, BenchMark XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab. and UltraView (760-500), UltraView with amplification (760-500 + 760-080) or OptiView (760-700) as detection systems. Using these protocol settings, 114 of 114 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP35**, product no. **RMA-0620**, Fuzhou Maixin, Titan-Stainer:
One protocol with an optimal result was based on HIER in DNS buffer (efficient heating time 18 min. at 99°C), 30 min. incubation of the primary Ab and Titan Super (TT-0805) as detection system.

rmAb clone **C9E15**, product no. **CCR-0343**, Celnovte, CNT330-Stainer:
One protocol with an optimal result was based on HIER using Tris-EDTA pH 9 (efficient heating time 20 min. at 100°C), 30 min. incubation of the primary Ab. and MicroStacker Flex Polymer (SD5300) as detection system.

rmAb clone **458G4A1**, product no. **PA285**, Abcarta, FAIP/48T-Stainer:
One protocol with an optimal result was based on HIER using EDTA/ER2 pH 9 (efficient heating time 20 min. at 100°C), 15 min. incubation of the primary Ab. and abcarta-HRP Polymer (PS300) as detection system.

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 CD4 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb 4B12 **IR649**	93% (13/14)	57% (8/14)	93% (14/15)	67% (10/15)
Leica BOND III mAb 4B12 **PA0427**	77% (10/13)	8% (1/13)	44% (4/9)	22% (2/9)
VMS Ultra/XT/GX rmAb SP35 **790-4423**	100% (31/31)	98% (30/31)	97% (111/114)	91% (104/114)

* 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 fouth NordiQC assessment for CD4, the prevalent features of insufficient staining results were characterized by a generally too weak/false negative staining reaction of the cells expected to be demonstrated, often in combination with a poor-signal-to noise ratio. Too weak or false negative staining results were observed in 64% of the insufficient results (43/67). Virtually all laboratories were able to detect CD4 in high-level antigen expressing cells as normal T-helper/inducer cells in the tonsil. In contrast the demonstration of CD4 in low-level antigen expressing cells as e.g., the germinal centre macrophages in the tonsil, Kupffer cells, endothelial cells in the liver sinusoids and of diagnostic importance, the neoplastic cells of the T-cell lymphoma was more challenging and could only be obtained with optimally calibrated protocols.
Poor-signal-to noise ratio and/or excessive background reactions were observed in 34% (23/67) of the insufficient results and as mentioned above often seen in combination with weak/false negative staining reactions. This observation was primarily related to staining on the Omnis platform (Dako/Agilent) in which 87% (20/23) of the protocols gave this atypical staining pattern. The primary cause for this problem is likely related to poor lots of the HRP Envision Flex reagents (Dako/Agilent), that has been on the marked since November-December 2022. Dako/Agilent is aware of this problem and has not yet been solved by up-loading date of this report.

Used within laboratory developed assays (LD) as concentrate or Ready-to-Use formats (RTU), the mAb clone 4B12 and the rmAb clone SP35 were the most widely used antibodies for demonstration of CD4 and applied by 93% (309/332) of the laboratories (see Table 1). Used as concentrated format within LD-assays, the rmAb clone SP35 provided the highest proportion of sufficient and optimal results, 92% (33/36) and 58% (21/36), respectively. As shown in Table 2, the rmAb clone SP35 provided optimal results on all main fully automatic platforms. Virtually all protocols assessed as optimal were based on efficient HIER in an alkaline buffer (21/21), the primary antibody was carefully calibrated in the dilution range of 1:10-1:50 (20/21) in combination with a sensitive 3-step detection system (18/21) e.g., EnVision Flex+(Dako/Agilent) or OptiView (Ventana/Roche). The main cause for an insufficient staining result was primarily related to the use of too short efficient HIER time in CC1 on the Benchmark Ultra platform (Ventana/Roche).

Protocols based on the mAb clone 4B12 as concentrate, provided a low pass rate of 54% (14/26) of which only 8% (2/26) being optimal. The performance is comparable to the results obtained in the previous run 44 for CD4, and thus, the antibody seems challenging from a technical point of view. As described in the former run, the performance of mAb clone 4B12 is influenced by the chosen IHC-instrument and in this assessment run 67, all CD4 NQC slides (5/5) stained on an Omnis were assessed as insufficient, although applying protocol settings providing a high analytical sensitivity such as efficient HIER in TRS pH 9, a dilution range of the primary antibody between 1:20-1:100 and EnVision Flex+ as the detection system. This pattern was also observed for the RTU format IR649 (Dako/Agilent), developed/validated for the Autostainer (Dako/Agilent), and based on the same clone (see below). In comparison and using similar protocol settings on the Autostainer (Dako/Agilent), the pass rate was 80% (4/5) of which 20% (1/5) were given an optimal mark. Surprisingly, this optimal protocol was based on HIER in TRS pH 6.1, normally providing lower analytical sensitivity of the assay.

For participants using the mAb 4B12 as concentrate on the Leica Bond-III platform, the pass rate was 67% (10/15) but only one protocol provided an optimal result. No parameters could be identified discriminating optimal from less successful performances (including insufficient staining results).
In the previous NordiQC assessment of CD4 (Run 44, 2015), six laboratories used the mAb clone 4B12 as concentrate on the BenchMark Ultra (Ventana/Roche) - all providing an insufficient staining result. Encouraging, and seen in this assessment, none of the Benchmark Ultra users applied this clone within LD-assays - likely shifting towards more robust clones either as concentrates or RTU formats. In general, and for participants struggling to optimize the CD4 assay, the continuously inferior performance of the mAb clone 4B12 on the fully automated platforms (all major vendors) should inspire laboratories to substitute the mAb 4B12 with a more robust clone as e.g., rmAb SP35 or EP204.

79% (261/332) of the laboratories used a RTU format for detection of CD4. This is a significant increase compared to the former run 44 in which 63% (147/234) of the participants applied a RTU format. In this assessment, the RTU system from Ventana/Roche 790-4423 based on the rmAb clone SP35 and developed for the Benchmark XT/Ultra platforms provided the highest proportion of sufficient and optimal results and was especially superior in regard of the portion of optimal results compared to the level of other RTU systems from all main vendors (see Table 3). Using vendor recommended protocol settings, all protocols (31/31) provided a sufficient result of which 98% (30/31) were optimal. Laboratory modified protocol settings (typically adjusting HIER time, incubation time of the primary Ab and/or choice of detection system) could be used with almost same success rate (see Table 3). Using this system, only three protocols gave an insufficient result and all were based on inappropriate antigen retrieval settings – no or too short HIER time (16 minutes) in CC1.

Ten laboratories used the RTU format 104R-17/18 (Cell marque) also based on the rmAb clone SP35, providing a pass rate of 90% (9/10) of which 60% (6/10) were given an optimal mark. Optimal results could be obtained on all fully automated instruments from the most common vendors.

The RTU system from Dako/Agilent IR649 based on the mAb clone 4B12, calibrated/validated for the Dako Autostainer platform, also provided a high pass rate of 93% (13/14) using vendor recommended protocol settings (see Table 3). However, the proportion of optimal results was significant lower (57%, 8/14) compared to the Ventana/Roche RTU system 760-4423. Applying laboratory modified protocol settings the proportion of optimal results increased to 67% (10/15) but no protocol parameters could be identified explaining for this improvement.
A significant proportion of the laboratories used this RTU system IR649 on an Dako Omnis (Dako/Agilent) – 45% (28/62) of all participants using Omnis as IHC platform. None (28/28) of these participants were able to obtain a sufficient staining result and as mentioned above, the performance of mAb clone 4B12 is influenced to the chosen staining device, especially the fully automated platforms Omnis and Benchmark Ultra/XT. It must be emphasized that the RTU format IR649 based on mAb clone 4B12 has not been calibrated/validated to the Omnis instrument, and thus, it is highly recommended that laboratories substitute this RTU format with a robust clone working on this particular platform. Importantly, laboratories should initiate a validation process no matter which solution is taken as there are at present no "true plug and play CD4 RTU system" for the Omnis instrument.

Within the RTU family for CD4, 8% (22/261) of the laboratories used the RTU product PA0427 (Leica Biosystems) based on the mAb clone 4B12 on the BOND III instrument. As shown in Table 3, and applying vendor recommended protocol settings, the proportion of sufficient results was low (77%) and only 8% (1/13) being optimal. Using laboratory modified protocol settings, typically prolonging HIER time in BERS2 and/or incubation time in primary antibody, the proportion of sufficient results declined to 44% (4/9). These results indicate that the mAb clone 4B12 also is challenging on this particular platform and is in line with the results obtained for the LD-assay in this assessment (see above). Substituting this RTU assay with a more robust clone as the rmAb clones SP35 or EP204, either as concentrate or RTU format, require that the protocol is meticulously validated especially in regard of analytical sensitivity as Bond Refine by nature acts as a 2-step detection system for these specific antibody clones (only enhances primary mouse monoclonal antibody reactions).

This was the fourth assessment of CD4 in NordiQC (see Graph 1). The pass rate was similar compared to results obtained in the previous run 44 (2015). In this assessment, the performance of the Ventana/Roche RTU system 760-4423 based on the rmAb clone SP35 was superior to all other RTU systems. The main cause for insufficient results in this assessment was related to the use of the mAb clone 4B12, both as concentrate and RTU formats, on the fully automated instruments Omnis and BOND III. Grouped together, 69% (46/67) of all insufficient results were related to the performance of the mAb clone 4B12 on these two platforms.
Importantly, the primary Abs must be careful calibrated according to the expected antigen level of the recommended control material (see below).

Controls
Tonsil is recommended as positive and negative tissue controls for CD4. In the tonsil, the protocol must be calibrated to provide a distinct and strong membranous staining reaction of all helper/inducer T-cells. Germinal centre macrophages should at least display a weak and distinct staining reaction. No staining reaction should be seen in B-cells and squamous epithelial cells of the tonsil.
As a supplement to tonsil, liver tissue can be used in which the Kupffer cells and endothelial cells in the liver sinusoids must display an at least moderate, but distinct staining reaction. Hepatocytes should be negative.

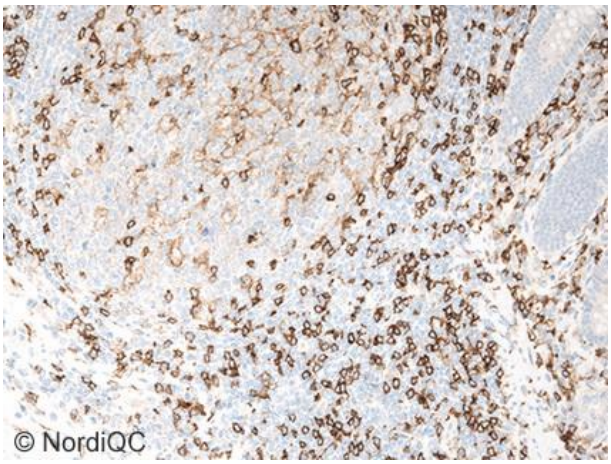


Fig. 1a (x200)
Optimal staining reaction for CD4 of the appendix applying the RTU assay IR649 (Autostainer, Dako/Agilent) based on the mAb clone 4B12, following vendor recommended protocol settings based on HIER in TRS (3-in-1) pH 9 and Envision FLEX+ as detection system.
All T-helper/inducer cells show a strong and distinct membranous staining reaction. The germinal centre macrophages display a weak to moderate staining intensity. No staining reaction was observed in B-cells and epithelial cells of the appendix. Same protocol used in Figs. 2a - 4a.

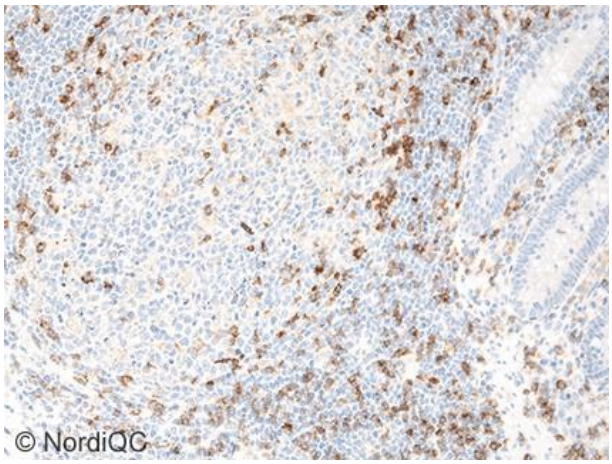


Fig. 1b (x200)
Insufficient staining reaction for CD4 of the appendix applying the same RTU system as in Fig. 1a, but used on the fully automated instrument Omnis (Dako/Agilent) with similar protocol settings as in Fig 1a - same protocol used in Figs. 2b - 4b.
The staining intensity is significantly reduced in T-helper/inducer cells and germinal centre macrophages are false negative or only faintly demonstrated. This antibody clone provides too low analytical sensitivity on this particular platform (see description above) and should prompt laboratories to substitute to a robust primary Ab as e.g., the rmAb clones SP35 or EP204 - compare with Fig. 2a-4b.

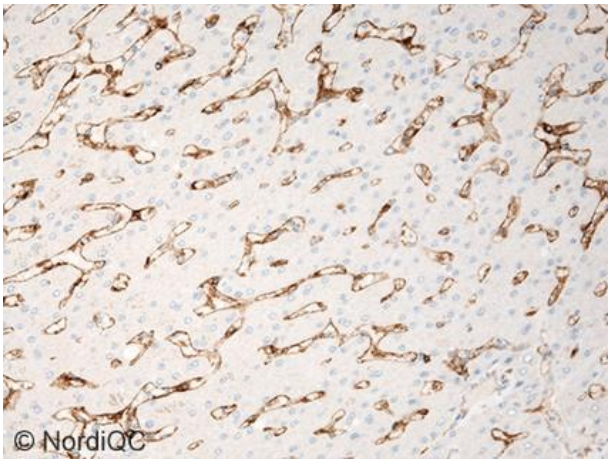


Fig. 2a (x200)
Optimal staining reaction for CD4 of the liver using same protocol as in Fig. 1a. The Kupffer cells and endothelial cells in the liver sinusoids show a moderate and distinct membranous staining reaction, whereas hepatocytes are negative.

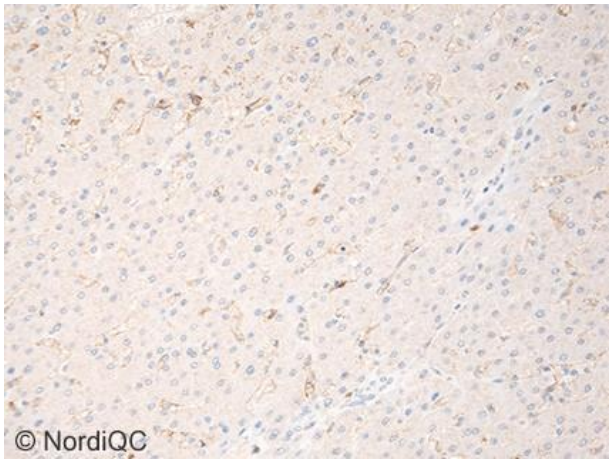


Fig. 2b (x200)
Insufficient staining reaction for CD4 of the liver using same protocol as in Fig. 1b. The Kupffer cells and endothelial cells in the liver sinusoids are false negative – compare with Fig. 2a. In addition, the hepatocytes display a faint to weak background staining likely related to lot problems with the HRP EnVision reagent (Dako/Agilent) (see description above). This "background reaction pattern" is well-known to Dako/Agilent and has been solved by 1. March 2023. The overall low analytical sensitivity of the protocol impacted the performance in the T-cell lymphoma as illustrated in Figs. 4a-4b.

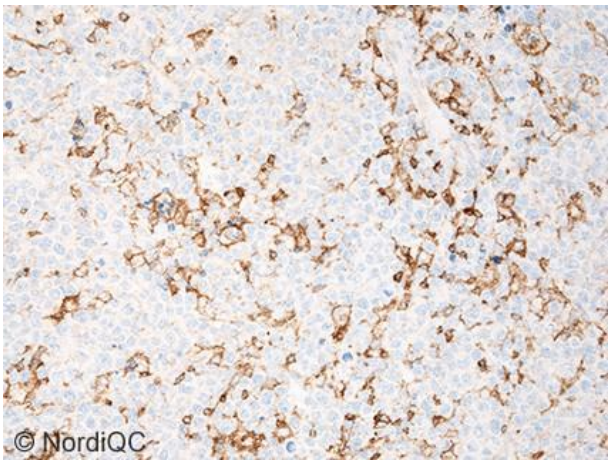


Fig. 3a (x200)
Optimal staining reaction for CD4 of the DLBCL using same protocol as in Figs. 1a and 2a. All the neoplastic B-cells are as expected negative. Subsets of macrophages and normal T-helper/inducer cells are distinctively demonstrated intermingling between the neoplastic B-cell.

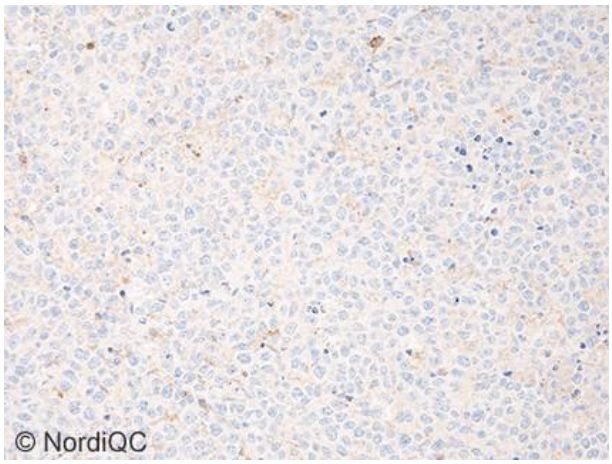


Fig. 3b (x200)
Insufficient staining reaction for CD4 of the DLBCL using same protocol as in Figs. 1b and 2b. The neoplastic B-cells are negative as expected, but the protocol provided too weak staining intensity and virtually all macrophages and T-helper/inducer cells are false negative – compare with Fig. 3a.

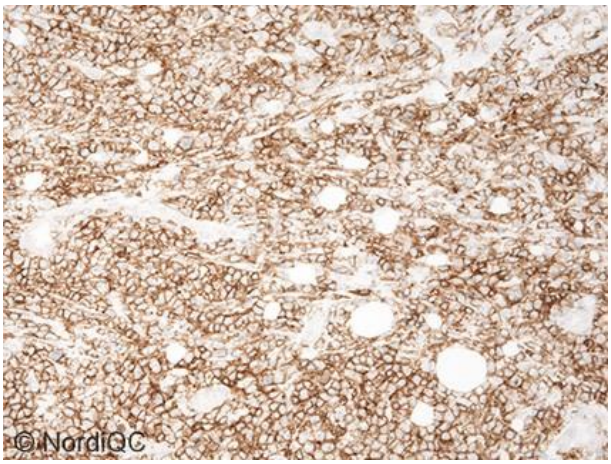


Fig. 4a (x200)
Optimal staining reaction for CD4 of the T-cell lymphoma, using same protocol as in Figs. 1a – 3a. All neoplastic T-cells cells display a moderate to strong, but distinct membranous staining reaction.

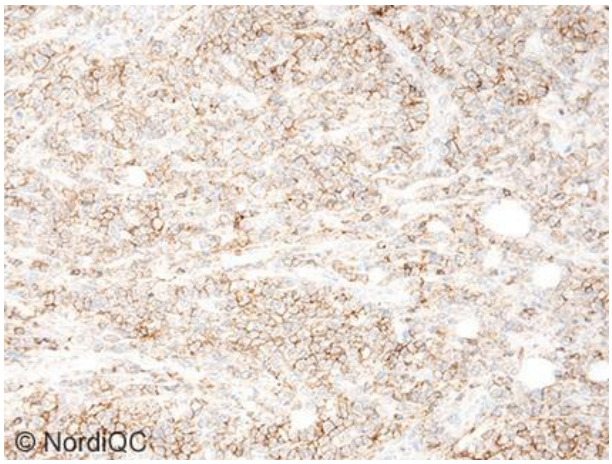


Fig. 4b (x200)
Insufficient staining reaction for CD4 of the T-cell lymphoma, using same protocol as in Figs. 1b – 3b. The vast majority of the neoplastic T-cells show a significantly reduced staining intensity or are false negative – compare with Fig. 3a.

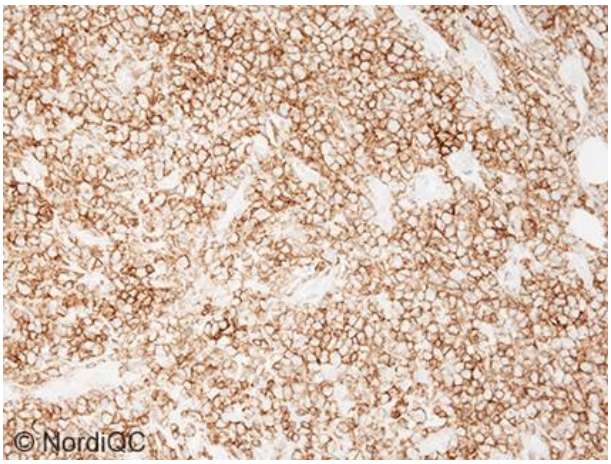


Fig. 5a (x200)
Optimal staining reaction for CD4 of the T-cell lymphoma using the RTU assay 790-4423 (BenchMark Ultra/XT, Ventana/Roche) based on the rmAb clone SP35, applying vendor recommended protocol settings based on HIER in CC1 (64 min. at 95°C), 32 min. incubation time in primary Ab and UltraView as the detection system. The protocol gave the same reaction pattern in all tissue cores as illustrated in Figs. 1a – 4a. This RTU system provided superior results in this assessment, also compared to LD-assays and other RTU formats/systems (see description above).

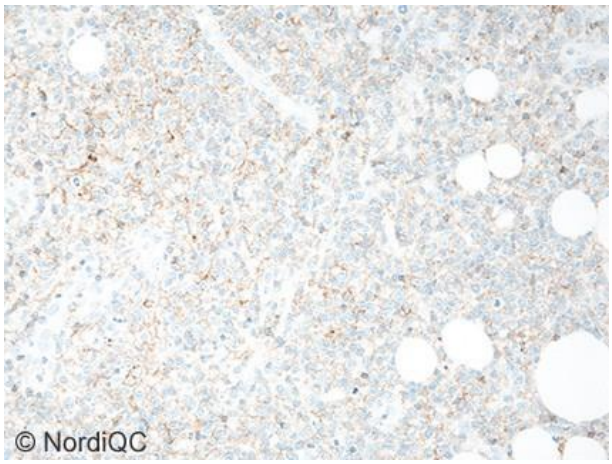


Fig. 5b (x200)
Insufficient staining reaction for CD4 of the T-cell lymphoma using the same RTU product as in Fig. 5a, but with reduced HIER time in CC1 (16 min. at 95°C), reduced incubation time in primary Ab (8 min.) and OptiView as detection system. Although the protocol was based on the sensitive detection system OptiView, the analytical sensitivity of the "overall system" is too low and parameters decreasing HIER time and incubation in primary antibody impacted performance in a negative direction. The proportion and intensity of stained neoplastic T-cells are significantly reduced, and most cells are false negative – compare with Fig. 5a.

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