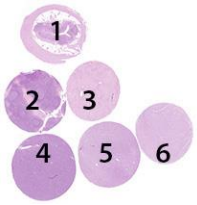
Assessment Run 59 2020

CD45 (Leucocyte Common Antigen)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD45 used to identify hematological/lymphoid neoplasms in the diagnostic workup for tumors of unknown primary origin. Relevant clinical tissues, both normal and neoplastic, were selected for a broad spectrum of antigen densities for CD45 (see below).

Material
The slide to be stained for CD45 comprised:



1. Appendix, 2. Tonsil, 3. Liver, 4. Burkitt Lymphoma (BL), 5. Diffuse Large B-Cell Lymphoma (DLBCL), 6. Malignant Melanoma (MM).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD45 staining as optimal included:

- A strong, predominantly membranous staining reaction of virtually all normal lymphocytes/histiocytes in all specimens tested. In tonsil, both the B- and T-cells should be distinctively demonstrated.
- An at least weak to moderate, distinct staining reaction of virtually all Kupffer cells in the liver.
- A strong, distinct membranous staining reaction of all neoplastic B-cells in the BL.
- A moderate to strong, distinct membranous staining reaction of virtually all neoplastic B-cells in the DLBCL
- No staining reaction in other cellular structures including columnar epithelial cells and smooth muscle cells of lamina muscularis propria of the appendix, squamous epithelial cells of the tonsil, hepatocytes of the liver and the neoplastic cells of the MM.

Participation

Number of laboratories registered for CD45, run 59	365
Number of laboratories returning slides	297 (81%)

The number of laboratories returning slides has decreased in this run 59 compared to previous assessments, due to the COVID-19 pandemic. All slides returned after the assessment will be assessed, and receive advice if the result is insufficient, but will not be included in this report.

Results
296 laboratories participated in this assessment. One laboratory used an inappropriate antibody. 277 (94%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody and/or too short incubation time
- Omission of Heat Induced Epitope Retrieval (HIER)
- Insufficient HIER (too short heating time)
- Unexplained technical issues

Performance history
This was the third NordiQC assessment of CD45. The overall pass rate increased significantly compared to the result obtained in recent run 37, 2013 (see Table 2).

Table 2. **Proportion of sufficient results for CD45 in the three NordiQC runs performed**

	Run 15 2005	Run 37 2013	Run 59 2020
Participants, n=	80	205	296
Sufficient results	86%	82%	94%

Conclusion
The mAb clones 2B11+PD7/26, PD7/26/16+2B11, X16/99, RP2/18 and the rmAb clone GR009 are all recommendable antibodies for demonstration of CD45. Efficient HIER in either an alkaline or citric based buffer and precise calibration of the primary Ab were the main prerequisites for an optimal result. In general, the Ready-To-Use (RTU) formats contributed positively to the high proportion of sufficient results. The RTU systems based on the mAb clone cocktail 2B11+PD7/26 from the two major vendors, Dako and Ventana, provided superior results and all protocols applied in compliance with the package inserts, were (36/36) assessed as optimal. Tonsil and liver are recommended as positive and negative tissue controls for demonstration of CD45. In tonsil, all the lymphocytes/histiocytes must display a strong and distinct membranous staining reaction whereas the squamous epithelial cells must be negative. In liver, the Kupffer cells must at least show a weak to moderate staining reaction while hepatocytes should be negative.

Table 1. **Antibodies and assessment marks for CD45, run 59**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clones **2B11+PD7/26**	89 6 1 1 1 1	Agilent/Dako Cell Marque Diagnostic Biosystem Zeta Corporation Zytomed Systems Linaris	73	19	6	1	93%	74 %
mAb clones **PD7/26/16+2B11**	5	Thermo S./LabVision	3	1	1	0	80%	60%
mAb clone **X16/99**	4	Leica/Novocastra	0	1	2	1	-	-
mAb clone **BS65**	1	Nordic BioSite	0	1	0	0	-	-
Ready-To-Use antibodies								OR[2]
mAb clones **2B11+PD7/26 GA751 (VRPS)**[3]	23	Agilent/Dako	23	0	0	0	100%	100%
mAb clones **2B11+PD7/26 GA751 (LMPS)**[4]	27	Agilent/Dako	23	4	0	0	100%	85%
mAb clones **2B11+PD7/26 IR/IS751 (VRPS)**[3]	6	Agilent/Dako	6	0	0	0	100%	100%
mAb clones **2B11+PD7/26 IR/IS751(LMPS)**[4]	18	Agilent/Dako	17	0	0	1	94%	94%
mAb clones **2B11+PD7/26 760-4279 (VRPS)**[3]	7	Ventana/Roche	7	0	0	0	100%	100%
mAb clones **2B11+PD7/26 760-4279 (LMPS)**[4]	36	Ventana/Roche	32	4	0	0	100%	89%
mAb clones **2B11+PD7/26 145M-90/97/98**	6	Cell Marque	5	1	0	0	100%	83%
mAb clones **2B11+PD7/26 MAD-002066QD**	2	Master Diagnostica	0	2	0	0	-	-
mAb clones **2B11+PD7/26 PM/IP016**	2	Biocare Medical	1	1	0	0	-	-
mAb clones **PD7/26/16+2B11 AM111**	1	Biogenex	0	1	0	0	-	-
mAb clone **X16/99 PA0042 (VRPS)**[3]	5	Leica Biosystems	3	1	1	0	80%	60%
mAb clone **X16/99 PA0042 (LMPS)**[4]	4	Leica Biosystems	1	3	0	0	-	-

mAb clone **RP2/18 760-2505 (VRPS)**[3]	3	Ventana/Roche	0	0	2	1	-	-
mAb clone **RP2/18 760-2505 (LMPS)**[4]	45	Ventana/Roche	36	6	3	0	93%	80%
rmAb clone **GR009 8271-C010**	2	Sakura Finetek	2	0	0	0	-	-
Total	296		232	45	15	4	-	
Proportion			79%	15%	5%	1%	94%	

1) Proportion of sufficient stains (optimal or good) (≥5 asessed protocols)
2) Proportion of Optimal Results (OR) (≥5 asessed protocols).
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 CD45, Run 59
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb **2B11+PD7/26**: Protocols with optimal results were typically based on HIER using an alkaline buffer as Cell Conditioning 1 (CC1) (Ventana) (37/51) *, Target Retrieval Solution (TRS) High pH (3-in-1) (Dako) (10/14), Bond Epitope Retrieval Solution 2 (BERS2) (Leica) (10/11), NovoCastra Epitope Retrieval Solution pH 9 (Leica) (1/1), Montage EDTA Antigen Retrieval Solution (Diagnostic Biosystems) (1/1), Retrieval Universal (Aptum) (1/1) or TRIS-EDTA HIER Solution pH 9 (Zeta Corporation) (1/1) as retrieval buffer. Eleven laboratories obtained an optimal mark performing HIER in acidic buffer using Bond Epitope Retrieval Solution 1 (BERS1) (Leica) (8/8), Target Retrieval Solution (TRS) Low pH (3-in-1) (Dako) (1/2), Cell Conditioning 2 (CC2) (Ventana) (1/3) or Citrate buffer pH 6 (Zytomed Systems) (1/1). The mAb was typically diluted in the range of 1:50 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 70 of 71 (99%) laboratories produced a sufficient staining (optimal or good). One laboratory obtained an optimal mark performing no pre-treatment at all.
* (number of optimal results/number of laboratories using this buffer)

mAb **PD7/26/16+2B11**: Protocols with optimal results were based on HIER using BERS2 (Leica) (1/1), CC1 (Ventana) (1/2) or TRIS-EDTA pH 9 (1/1) as retrieval buffer. The mAb was diluted in the range of 1:400 – 1:1.000 depending on the total sensitivity of the protocol employed.

Table 3. **Proportion of optimal results for CD45 for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibody	**Dako Autostainer Link/Classic**		**Dako Omnis**		**Ventana BenchMark GX /XT/ Ultra**		**Leica 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 **2B11+PD7/26**	3/5** (60%)	1/1	4/4	-	34/40 (85%)	1/2	6/7 (86%)	6/6 (100%)

* 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 clones **2B11+PD7/26,** product no. **GA751,** Dako, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20-30 min. at 97°C), 10-20 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823 + GV821) as detection system. Using these protocol settings, 28 of 28 (100%) laboratories produced a sufficient result (optimal or good). Applying vendor recommended protocol settings (VRPS), all protocols (23/23) provided an optimal result (see Table 1).

mAb clones **2B11+PD7/26,** product no. **IS/IR751,** Dako, Autostainer+ /Autostainer Link:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 95-97°C), 10-20 min. incubation of the primary Ab and EnVision Flex (K8000/K8002) as detection system. Using these protocol settings, 10 of 11 (91%) laboratories produced a sufficient result. Applying VRPS, all protocols (6/6) provided an optimal result (see Table 1).

mAb clones **2B11+PD7/26,** product no. **760-4279**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 24 of 24 (100%) laboratories produced a sufficient result. Applying VRPS, all protocols (7/7) provided an optimal result (see Table 1).

mAb clone **RP2/18,** product no. **760-2505**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 32-64 min. at 95-100°C), 16-36 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings, 32 of 33 (97%) laboratories produced a sufficient result. Only 6% (3/48) of the laboratories applied VRPS of which none provided a sufficient result.

mAb clone **X16/99,** product no. **PA0042,** Leica, BOND III/BOND MAX:
Protocols with optimal results were typically based on HIER using BERS1 (efficient heating time 20 min. at 100°C), 15 min. incubation of the primary Ab and BOND Refine (DS9800) as detection system. Using these protocol settings, 4 of 5 (80%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 80% (4/5) and 60% (3/5) optimal.

rmAb clone **GR009,** product no. **8271-C010,** Sakura FineTek, Tissue-Tek Genie Advanced Stainer:
Protocols with optimal results were based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (efficient heating time 45-60 min. at 98°C), 30 min. incubation time of primary Ab and Tissue-Tek Genie Pro Detection Kit DAB (8826-K250) as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥ 5 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for CD45 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako Omnis mAb 2B11+PD7/26 **GA751**	100% (23/23)	100% (23/23)	100% (25/25)	88% (22/25)
Dako AS mAb 2B11+PD7/26 **IR751**	100% (6/6)	100% (6/6)	86% (6/7)	86% (6/7)
VMS Ultra/XT/GX mAb 2B11+PD7/26 **760-4279**	100% (7/7)	100% (7/7)	100% (36/36)	89% (32/36)
VMS Ultra/XT/GX mAb RP2/18 **760-2505**	(0/3)	(0/3)	93% (42/45)	80% (36/45)
Leica Bond III/MAX mAb X16/99 **PA0042**	80% (4/5)	60% (3/5)	(4/4)	(1/4)

* 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 were included.

Comments
In this third NordiQC assessment of CD45, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells expected to be demonstrated. Virtually all laboratories were able to stain CD45 in normal lymphocytes (all specimens), whereas demonstration of CD45 in Kupffer cells of the liver and the neoplastic cells of the DLBCL was more challenging and required a carefully calibrated protocol.

mAb clone cocktail 2B11+PD7/26 was the most widely used antibody for the demonstration of CD45 and was in total applied by 76% (226/296) of the participants. Used as a concentrate within a laboratory developed (LD) assay, the mAb gave an overall pass rate of 93% (92/99). As shown in Table 3, a high proportion of optimal results could be obtained on the three fully automated IHC platforms from Dako, Leica and Ventana. The most common cause of insufficient staining results was use of protocols with too low analytical sensitivity, typically using several inadequate parameter settings in combination as omission of HIER / inefficient HIER (too short time), too diluted and/or too short incubation time of the primary Ab.

Applying Optimal Protocol Settings (OPS) (see description) based on efficient HIER in either an alkaline or citric based buffer and using the antibody in the "optimal" dilution range 1:50-1:400, the proportion of sufficient results increased to 99% (70/71) of which 84% (60/71) were assessed as optimal. In comparison, and using exactly the same protocol settings except for utilizing the primary Ab in the dilution range 1:500-1:3200, the proportion of sufficient results declined to 81% (17/21) and only 48% (10/21) were optimal, stressing the importance of using a carefully calibrated primary Ab. In this assessment, the proportion of sufficient and optimal results were unaffected by type of the detection system applied (2-step versus 3 step multimer/polymer) as long as efficient HIER was performed and the titre of the primary Ab was carefully calibrated according to the level of analytical sensitivity of the assay in use. In this context, the IHC protocol must be able to demonstrate CD45 in cellular structures with both low- and high-level CD45 expression as Kupffer cells of the liver and lymphocytes of the tonsil, respectively.

Overall, Ready-To-Use (RTU) systems or RTU formats were used by 63% (187/296) of the participants. In this assessment, the RTU systems IR/IS751 or GA751 (Agilent/ Dako) and 760-4279 (Roche/Ventana) based on the mAb clone cocktail 2B11+PD7/26 provided superior results (see Table 1 and 4). Grouped together, all protocols (36/36) following Vendor Recommended Protocol Settings (VRPS) provided an optimal result. This result is very encouraging as it reflects how a "true plug and play system" should work. It was observed that a variety of protocol settings could be used for optimal performance, e.g. for the Agilent/Dako RTU variants, Flex (Dako) is recommended as detection system on the Autostainer and Flex+ (Dako) on the Omnis, but each parameter is optimally designed and tailored to the specific platform on which the assay was performed, emphasizing, that an assay must be calibrated within a system (e.g. platform, HIER, incubation time in primary Ab and choice of detection system). With this in mind, a significant proportion of laboratories tend to use e.g. RTU products from Agilent/Dako off-label on non-validated platforms and often from other vendors as BOND III (Leica) or Benchmark Ultra (Ventana). In general, this approach cannot be recommended although all protocols (13/13) were assessed as sufficient of which 92% (12/13) were optimal. As a minimum, it requires that the laboratories have thoroughly validated the RTU format on the In-House platform with the respective reagents needed to run the process.
A high proportion of sufficient and optimal results could also be obtained using Laboratory Modified Protocol Settings (LMPS), typically adjusting HIER, incubation time of the primary Ab or use of a non-recommended detection system (see Table 4).

The RTU system based on the mAb clone RP2/18, prod.no. 760-2505 (Roche/Ventana) was used by 16% (48/296) of the participants. As shown in Table 4, only 6% (3/48) of the protocols were based on VRPS, whereas 94% (45/48) of the laboratories applied LMPS. However, and as observed in the former run 37 (2013), if the protocol was performed according to the package insert, based on no retrieval, an incubation time of 16 min. in the primary Ab and iView or UltraView as the detection system, all laboratories (n=3) obtained an insufficient result. In total (Run 37 and Run 59), and applying VRPS, none (8/8) of the protocols could provide a sufficient result. In this assessment, a high proportion of sufficient and optimal results were obtained using LMPS, typically applying HIER in CC1 (efficient HIER time for 32-64 min at 95-100°C), prolonging incubation time in primary Ab (16-32 min.) and using OptiView/UltraView as the detection system. Using these protocol settings, 97% (28/29) were assessed as sufficient of which 83% (24/29) were optimal. For laboratories utilizing OptiView as the detection system, the pass rate was 100% (13/13) and all were assessed as optimal.

The RTU system PA0042 based on the mAb clone X16/22 gave an overall pass rate of 89% (8/9). In this run, and using VRPS based on HIER in BERS1 (20 min. at 99-100°), 15 min. incubation time in primary ab and Bond Refine as the detection system, the pass rate was 80% (4/5) of which 60% (3/5) were optimal (see Table 1). In general, this antibody provided slightly weaker intensity especially in the neoplastic B-cell of the DLBCL compared to assays based on e.g. the mAb clone cocktail 2B11+PD7/26. This reaction pattern was seen with all formats of this product (concentrate and RTU) and was not related to any other protocol parameters as e.g. HIER buffer (BERS1 versus BERS2). It is difficult to elucidate on this observation as the assays based on the mAb clone X16/22 provided very good results in the former run 37 applying similar protocol settings. However, it is known from a NordiQC reference laboratory that this antibody can be challenging. Used within a LD-assay, best performance was obtained applying HIER in modified citric based buffer as TRS Low pH (Dako) or Diva Decloaker (Biocare), a relative high concentration of the primary Ab (1:25-1:40) and a sensitive 3-step detection system.

This was the third assessment of CD45 in NordiQC and a pass rate of 94% was obtained, which is a significant increase compared to the result obtained in run 37, 2013 (see Table 2). The use of robust primary Abs (e.g. mAb 2B11+PD7/26) but also well calibrated RTU systems accounted for the superior performance obtained in this assessment.

Controls

Tonsil and liver are recommendable as positive and negative tissue controls. In tonsil, all lymphocytes (B- and T-cells) and histiocytes must display a strong distinct membranous staining reaction. Squamous epithelial cells should be negative. In liver, the Kupffer cells should show a weak to moderate staining reaction whereas hepatocytes must be negative. Scattered lymphocytes can be seen in the liver and must display strong, distinct membranous staining reaction.

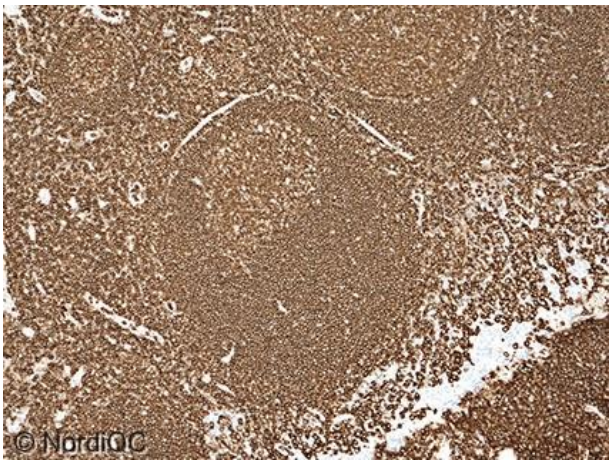


Fig. 1a (x100)
Optimal staining for CD45 of the tonsil using the RTU product 760-2505 based on the mAb clone RP2/18 (Ventana). The protocol was based on LMPS, applying efficient HIER in CC1, prolonged incubation time in primary Ab (32 min.) and use of the sensitive detection system OptiView - same protocol used in Figs. 2a - 4a. Virtually all lymphocytes/histiocytes show a distinct and strong membranous staining reaction while squamous epithelial cells are negative.

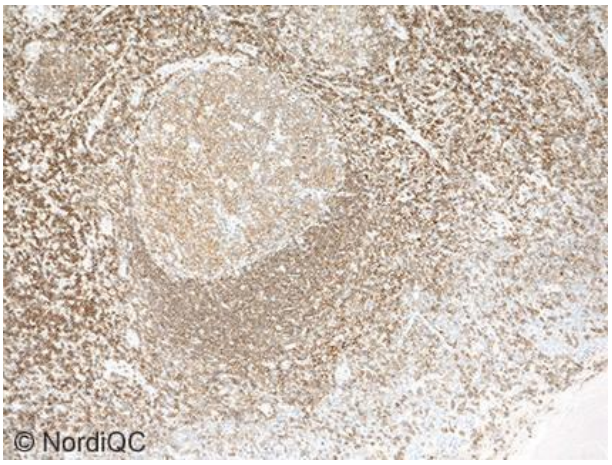


Fig. 1b (x100)
Insufficient staining for CD45 of the tonsil using the RTU product 760-2505 based on the mAb clone RP2/18 (Ventana). The protocol was based on VRPS, applying no pre-treatment, incubation time in primary Ab (16 min.) and UltraView as detection system - same protocol used in Figs. 2b – 4b. The intensity of the staining reaction is significantly weaker and the proportion of positive lymphocytes, both in the T-zones and germinal centres, is reduced - compare with Fig. 1a (same field).

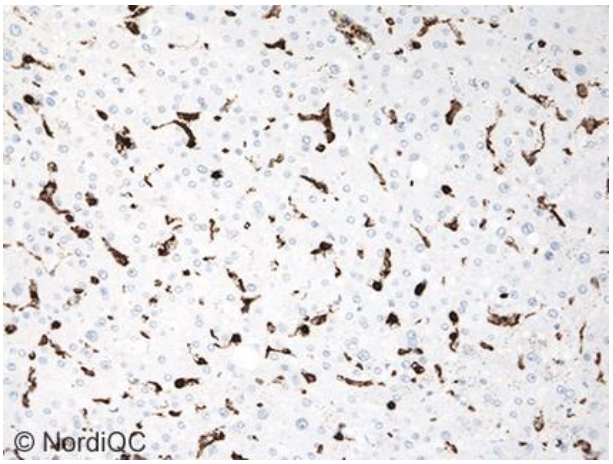


Fig. 2a (x200)
Optimal staining for CD45 in the liver using same protocol as in Fig. 1a. Virtually all Kupffer cells show a distinct, moderate to strong staining reaction, whereas hepatocytes are negative as expected.

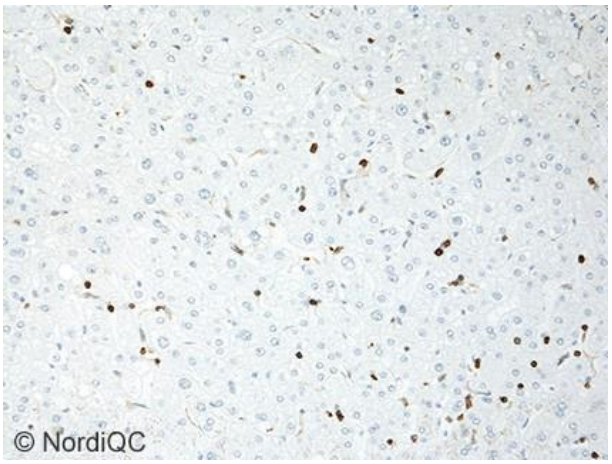


Fig. 2b (x200)
Insufficient staining for CD45 in the liver using same protocol as in Fig. 1b. Normal lymphocytes intermingling between hepatocytes are positive. The Kupffer cells are false negative or only faintly demonstrated - compare with Fig. 2a (same field).

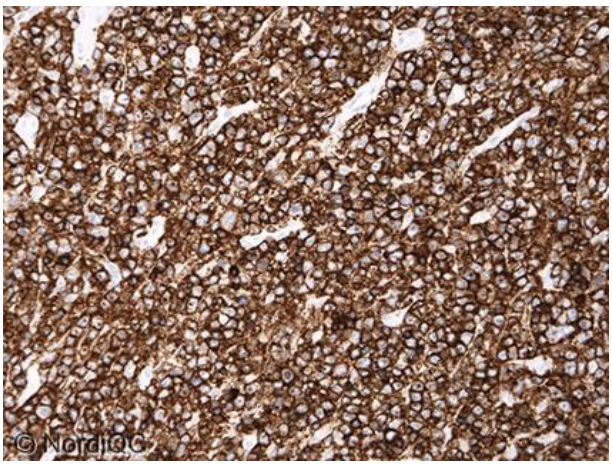


Fig. 3a (x200)
Optimal staining for CD45 of the DLBCL using same protocol as in Figs. 1a-2a. Virtually all the neoplastic B-cells display a strong membranous staining reaction.

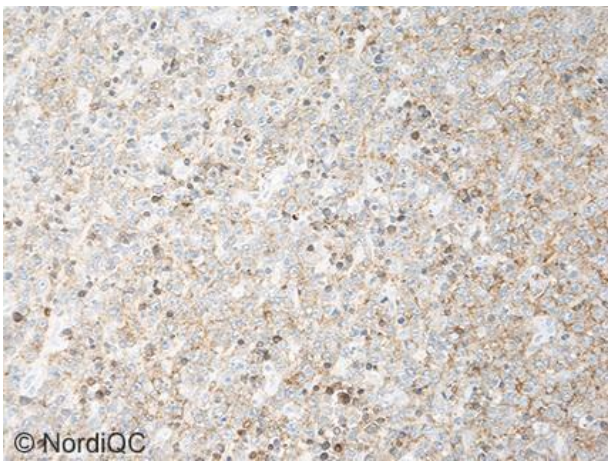


Fig. 3b (x200)
Insufficient staining for CD45 of the DLBCL using same protocol as in Figs. 1b-2b. A significant proportion of neoplastic B-cells are false negative or only display a faint to weak staining reaction - compare with Fig. 3a (same field).

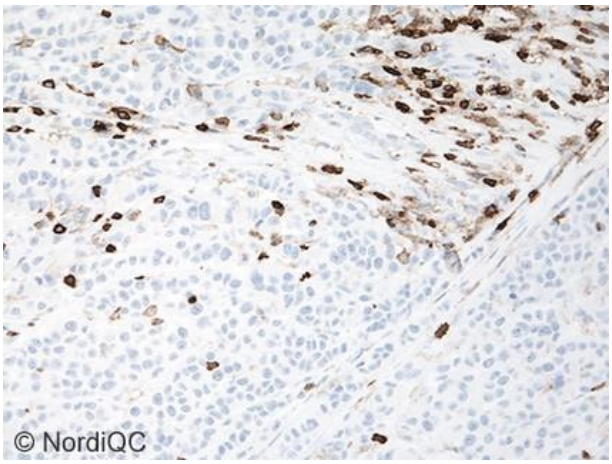


Fig. 4a (x200)
Optimal staining for CD45 of the MM using same protocol as in Figs. 1a-3a. Only normal lymphocytes/histiocytes are demonstrated, displaying a moderate to strong staining intensity, whereas the neoplastic cells of the MM are negative.

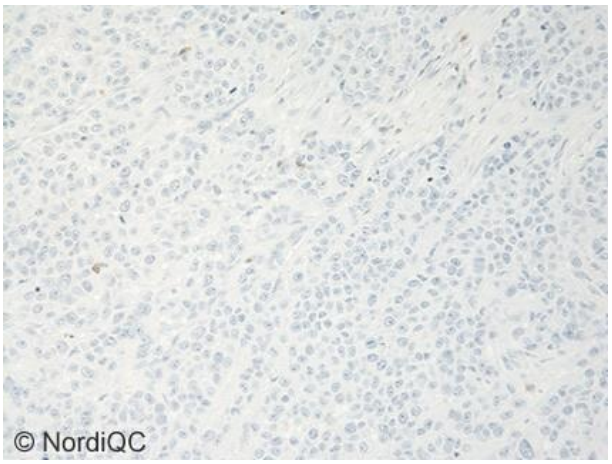


Fig. 4b (x200)
Insufficient staining for CD45 of the MM using same protocol as in Figs. 1b-3b. Although the neoplastic cells of the MM are negative as expected, the staining is too weak as lymphocytes/histiocytes intermingling between the neoplastic cells are false negative - compare with Fig. 4a (same field).

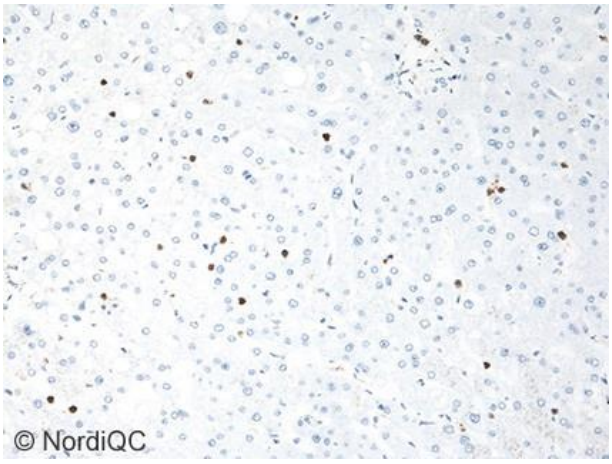


Fig. 5a (x200)
Insufficient staining for CD45 of the liver using the mAb clone cocktail 2B11+PD7/26 as a concentrate within a protocol providing a too low analytical sensitivity - too short HIER time (10 min. at 97°C) in TRS pH 9 (Dako), too low concentration of the primary Ab (1:3,200) and the polymer based detection system Flex+ (Dako) – same protocol used in Figs. 6a-7a. Only normal lymphocytes are demonstrated, whereas Kupffer cells are false negative. Although the protocol is based on the sensitive detection system Flex+, it must be emphasized, that all critical protocol parameters have to be optimized in order to provide the expected reaction pattern – compare with optimal results in Fig. 5b (same field).

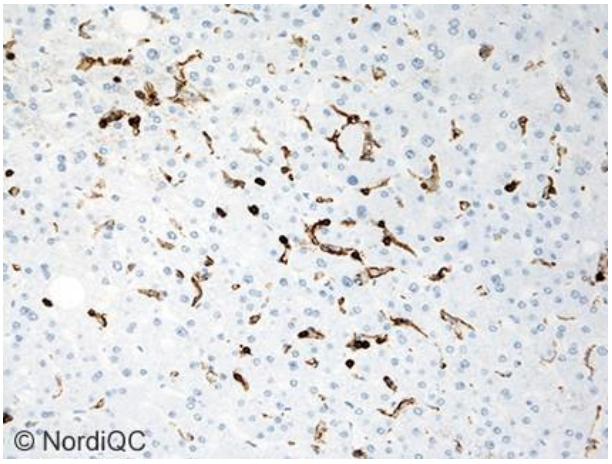


Fig. 5b (x200)
Optimal staining for CD45 of the liver using optimized protocol settings based on efficient HIER in TRS pH 9 (20 min. at 97°C), applying a carefully calibrated titre of the primary Ab (1:200) using Flex as the detection system (Dako) – same protocol used in Fig. 6b-7b. The Kupffer cells with the critical "low expression level -", show a weak to moderate staining reaction. This reaction pattern must be obtained to confirm appropriate level of analytical sensitivity, or otherwise, risking a misclassification of tumors of unknown origin - compare Figs. 5a-7b.

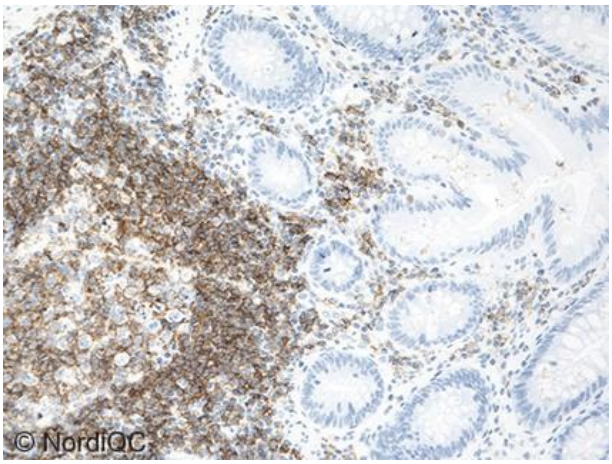


Fig. 6a (x200)
Insufficient staining for CD45 of the appendix using the same protocol as in Fig. 5a. The intensity and proportion of positive lymphocytes/histiocytes, especially of lamina propria mucosa, is significantly reduced - compare with results by an optimal protocol in Fig. 6b (same field).

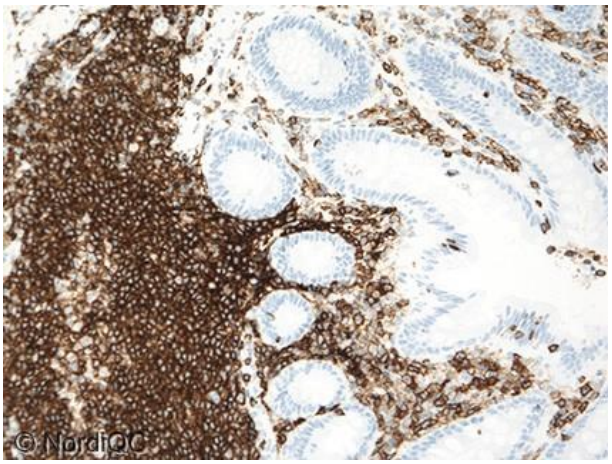


Fig. 6b (x200)
Optimal staining for CD45 of the appendix using the same protocol as in Fig. 5b. The vast majority of intraepithelial lymphocytes, lymphocytes/histiocytes of lymphoid aggregations (germinal centres) and in lamina propria mucosa, show a strong distinct membranous staining reaction.

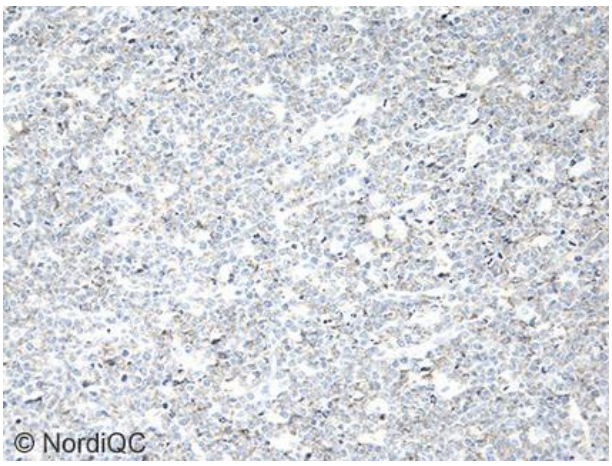


Fig. 7a (x200)
Insufficient staining for CD45 of the BL using same protocol as in Figs. 5a-6a. The vast majority of neoplastic B-cells are false negative or only faintly demonstrated - compare with an optimal protocol in Fig. 7b.

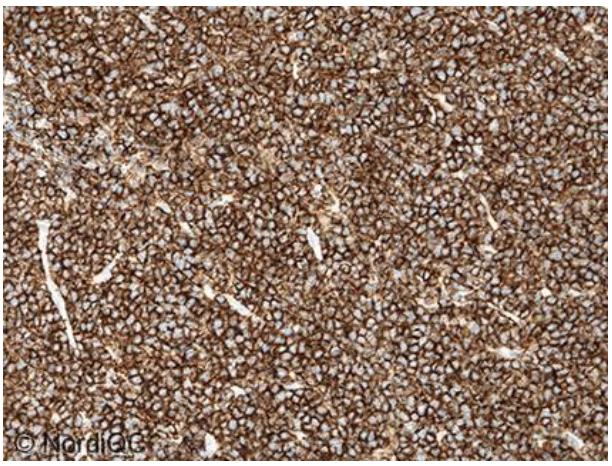


Fig. 7b (X200)
Optimal staining for CD45 of the BL using the same protocol as in Figs. 5b-6b. All neoplastic B-cells display a strong and distinct membranous staining reaction. All critical protocol parameters were optimally calibrated, and thus, optimal results could be obtained despite using a less sensitive detection system as Flex (Dako).

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