Assessment Run 37 2013

CD45

Leucocyte Common Antigen (LCA)

Material
The slide to be stained for CD45 comprised:

1. Tonsil, 2. Liver, 3. Brain, 4. B-CLL

All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a CD45 staining as optimal included:

- A moderate to strong and distinct predominantly membranous staining reaction of all lymphocytes in all four tissues tested. In the tonsil both the B- and T-cells should be distinctively demonstrated.
- An at least weak to moderate and distinct staining reaction of the Kupffer cells in the liver and the microglial cells of the brain.
- An at least weak to moderate predominantly membranous staining reaction of virtually all the neoplastic cells of the B-CLL
- No staining of squamous epithelial cells in the tonsil or hepatocytes in the liver.

214 laboratories participated in this assessment. 9 participants used an inappropriate antibody (CD45R0 and CD45RA). Of the remaining 205 laboratories 82% achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

Table 1. **Antibodies and assessment marks for CD45, run 37**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clones **2B11+PD7/26**	111 1 1	Dako Diagnostic Biosystems Zytomed	64	29	16	4	82 %	85 %
mAb clones **MEM28/MEM56 /MEM55**	1	Invitrogen	0	1	0	0	-	-
mAb clones **PD7/26/26+2B11**	3	Thermo/Neomarkers	0	1	2	0	-	-
mAb clone **X16/99**	9	Leica/Novocastra	6	2	0	1	89 %	100 %
rmAb clone **EP68**	1	Epitomics	0	0	0	1	-	-
Ready-To-Use Antibodies								
mAb clones **2B11+PD7/26 IS/IR751**	31	Dako	29	2	0	0	100%	100%
mAb clones **2B11+PD7/26 760-4279**	14	Ventana/Cell Marque	4	6	4	0	71 %	100 %
mAb clones **2B11+PD7/26 148M-98**	2	Cell Marque	2	0	0	0	-	-
mAb clones **2B11+PD7/26 N1514**	1	Dako	1	0	0	0	-	-

mAb clones **2B11+PD7/26 E005**	1	Linaris	0	0	1	0	-	-
mAb clones **2B11+PD7/26 MAD-004010QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clones **PD7/26/16+2B11 PM-016**	1	Biocare	0	1	0	0	-	-
mAb clone **RP2/18 760-2505**	21	Ventana	3	11	7	0	67 %	80 %
mAb clone **X16/99 PA0042**	6	Leica	6	0	0	0	100 %	%
Total	205		115	54	30	6	-	
Proportion			56 %	26 %	15 %	3 %	82 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of CD45, Run 37
Following protocol parameters were central to obtain an optimal staining:

Concentrated Abs
mAb clones **2B11+PD7/26**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution (TRS) pH 9 (3-in-1) (11/17)*, TRS pH 9 (Dako) (7/11), TRS low pH 6.1 (Dako) (3/3), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (9/10), BERS1 (Leica) (1/1), Cell Conditioning 1 (CC1; Ventana) (21/44), CC2 (Ventana) (1/3), Tris-EDTA/EGTA pH 9 (10/14) or Citrate pH 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:100-1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 87 of 102 (85 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **X16/99**: Protocols with optimal results were all based on HIER using either TRS low pH 6.1, (Dako)(1/1), BERS1 (Leica) (2/2), BERS2 (Leica) (1/2) or CC1 (Ventana) (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 of 7 (100 %) laboratories produced a sufficient staining.

The overall proportion of optimal staining results using these two concentrated Abs on the three most commonly used IHC stainer platforms is summarized in table 2

Table 2. **Optimal results for CD45 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	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 clones **2B11+PD7/26**	64 % 18/28**	100 % 3/3	48 % 21/44	33 % 1/3	90 % 9/10	100 % 1/1
mAb clone **X16/99**	-	100 % 1/1	100 % 2/2	-	50 % 1/2	100 % 2/2

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

Ready-To-Use Abs
mAb clone **2B11+PD7/26** (product.no. IS / IR751, Dako): Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min at 95-98°C), 10-20 min incubation in the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 31 of 31 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **2B11+PD7/26** (prod. no. 760-4279, Ventana/Cell Marque): Protocols with optimal results were all based on HIER using mild or standard Cell Conditioning 1, 32-52 min incubation in the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 7 of 7 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **RP2/18** (prod. no. 760-2505, Ventana): Protocols with optimal results were all based on HIER using mild or standard Cell Conditioning 1, 16-32 min incubation in the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 8 out of 10 (80 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **X16/99** (product. no. PA0042, Leica/Novocastra): Protocols with optimal results were based on HIER using BERS1 or BERS 2 (Bond, Leica), 15-30 min incubation in the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these settings 6 out of 6 (100 %) laboratories produced an optimal staining.

The most frequent causes of insufficient stainings were:
- Omission of HIER
- Too low concentration of the primary antibody

As in the previous NordiQC assessment of CD45 (run 15 2005) the prominent feature of an insufficient staining was a too weak or false negative staining of structures expected to be demonstrated. Virtually all laboratories were able to demonstrate CD45 in the normal B- and T-cells in the tonsil, whereas the demonstration of CD45 in the Kupffer cells and microglial cells were more challenging and required an optimally calibrated protocol.
Several antibodies could be used to obtain an optimal staining result. In general, use of HIER and a carefully calibrated Ab titre were the main parameters for an optimal staining result.
Omission of HIER was one of the main reasons for an insufficient result. All (n=8) protocols omitting HIER gave an insufficient staining result. In this context it has to emphasized, that misleading and imprecise guidelines regarding epitope retrieval and protocol set-up from many vendors still is a central issue and contributes to insufficient results. E.g. for the mAb clone RP2/18 provided as RTU, prod. no. 760-2505 from Ventana an overall pass rate of 67 % was seen. However, if the analysis 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=5) using these settings obtained an insufficient result. If the same RTU product was applied with an incubation time for 16-32 min., HIER in CC1 standard (64 min.) and UltraView as the detection system, all laboratories (n=4) obtained a sufficient result. Similar observations and inconsistent guidelines were seen for the mAb clones 2B11+PD7/26, Thermo/NeoMarkers. In the package insert omission of HIER is recommended if used with UltraVision LP (Thermo) but HIER is recommended if UltraVision Quanto (Thermo) is used.

Controls
As seen in this assessment tonsil, in combination with liver or brain, should be the preferred controls for CD45. In tonsil all lymphocytes in both the B- and the T-zones must show a moderate to strong and distinct continuous membranous staining reaction, whereas a patchy membranous staining reaction is a sign of inadequate protocol sensitivity. Tonsil will also verify that CD45, LCA was applied and not e.g. CD45RA, mAb clone 4KB5 for B-cells or CD45R0 mAb clone UCHL1 for T-cells. Squamous epithelial cells must be negative. In order to ensure a high sensitivity of the protocol, liver or brain tissue is highly recommended, as both Kupffer and especially microglial cells show a low CD45, LCA antigen expression and thus are the recommendable critical staining quality indicators for CD45, LCA. In the liver Kupffer cells must show an at least weak to moderate staining reaction, while the hepatocytes must be negative (except for lipofuscin, which can show a granular cytoplasmic staining reaction).

Effect of external quality assessment
This was the 2nd assessment of CD45 in the NordiQC quality programme. Similar pass rates were seen in the two runs, as shown in table 3.

Table 3. **Proportion of sufficient CD45 results in two NordiQC runs**

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

Conclusion
The mAb clones **2B11+PD7/26** and **X16/99** can both be recommended for the demonstration of CD45, LCA. Both the concentrated formats and Ready-To-Use systems for these two Abs gave a high proportion of sufficient results. HIER is mandatory for a sufficient result.
Tonsil in combination with liver is recommended as controls for CD45, LCA.
In tonsil all B- and T-cells must show strong and distinct membranous staining reaction, while Kupffer cells in liver or microglia in brain tissue must show an at least weak to moderate but distinct staining reaction. No staining should be seen in the squamous epithelial cells and hepatocytes.

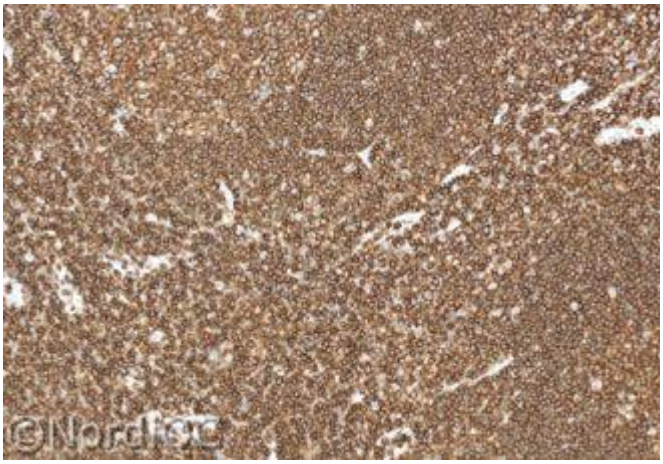

Fig 1a
Optimal CD45, LCA staining of the tonsil using the mAb clones 2B11+PD7/26 optimally calibrated and with HIER. Virtually all the B- and T-lymphocytes show a strong and distinct membranous staining reaction. No background staining is seen.
Also compare with Figs. 2a – 4a, same protocol.

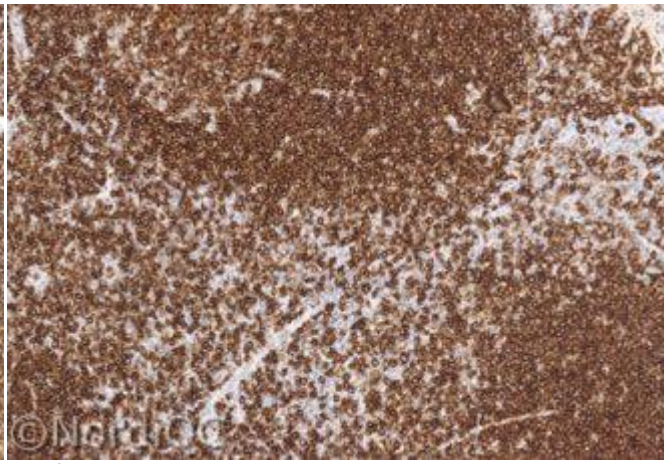

Fig 1b
Staining for CD45, LCA of the tonsil using the mAb clones 2B11+PD7/26 by protocol settings giving a too low sensitivity (too low concentration of the primary Ab) - same field as in Fig. 1a.
The vast majority of the B- and T-lymphocytes are demonstrated. However also compare with Figs. 2b – 4b, same protocol.

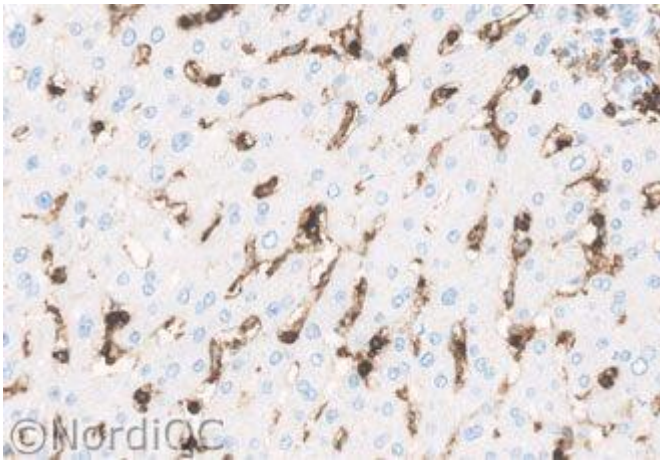

Fig 2a
Optimal CD45, LCA staining of the liver using same protocol as in Fig. 1a.
The lymphocytes show a strong staining reaction, while the Kupffer cells display a weak to moderate staining reaction. The liver cells are negative and no background staining is seen.

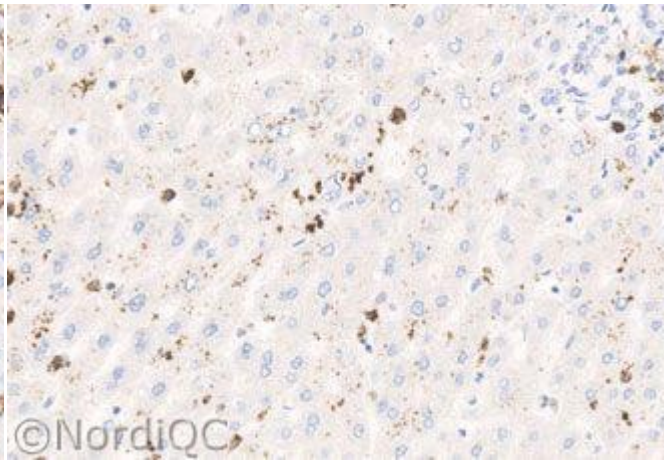

Fig 2b
Insufficient CD45, LCA staining of the liver using same protocol as in Fig. 1b – same field as in Fig. 2a.
Only lymphocytes are demonstrated and the Kupffer cells with a low CD45 expression are false negative.

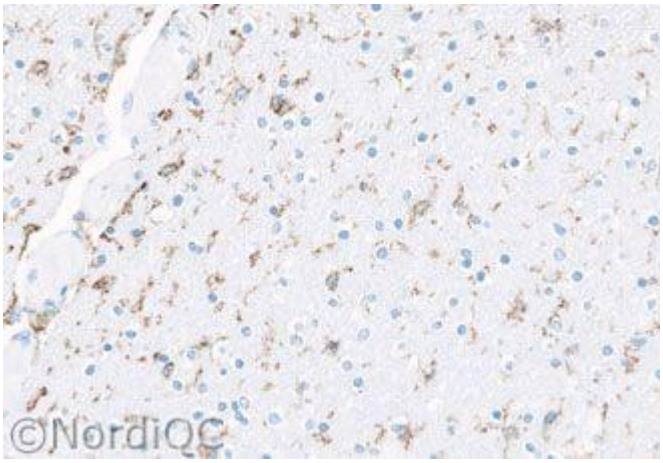

Fig 3a
Optimal CD45, LCA staining of the brain using same protocol as in Figs. 1a & 2a.
The microglial with a low CD45 expression are distinctively demonstrated and no background staining is seen.

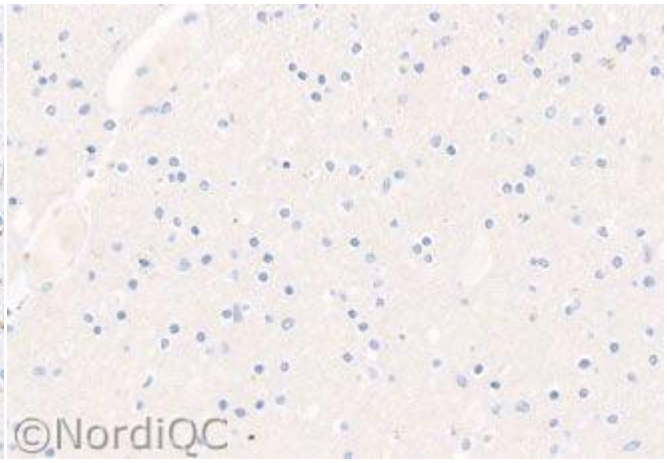

Fig 3b
Insufficient CD45, LCA staining of the brain using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The microglial cells are false negative.

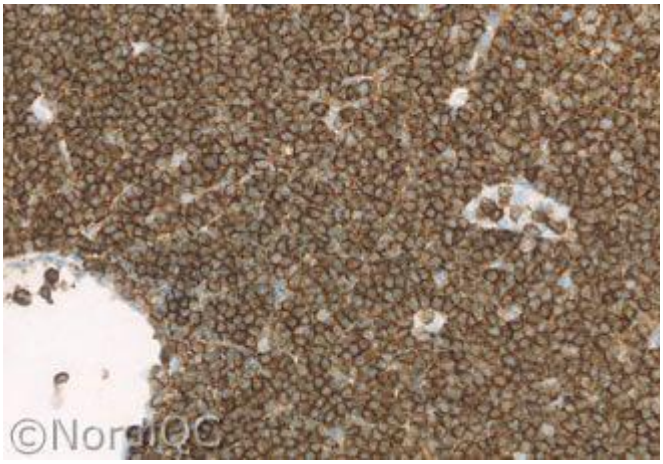

Fig 4a
Optimal CD45, LCA staining of the B-CLL using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong and distinct membranous staining reaction.
No background staining is seen.

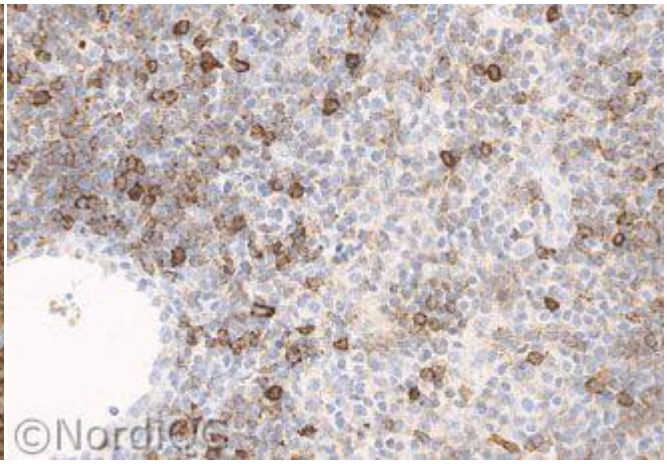

Fig 4b
Insufficient CD45, LCA staining of the B-CLL using same protocol as in Figs. 1b - 3b. – same field as in Fig. 4a.
The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 4a.

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