

**Material**

The slide to be stained for CD19 comprised:

1. Appendix, 2. Tonsil, 3. Diffuse large B-cell lymphoma (DLBCL), 4. Precursor-B-Acute lymphatic leukaemia (Pre-B-ALL), 5. B-Chronic lymphatic leukaemia (B-CLL), 6. Plasmacytoma.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD19 staining as optimal included:

- A strong, predominantly membranous staining of the mantle zone B-cells, the germinal centre B-cells, the interfollicular B-cells and follicular dendritic cells in the tonsil and the appendix. A weak staining in normal plasma cells is expected in the tonsil and the appendix.
- A moderate to strong membranous staining of virtually all the neoplastic cells of the DLBCL.
- A moderate to strong membranous staining of virtually all the neoplastic cells of the B-CLL.
- A moderate to strong membranous staining of virtually all the neoplastic cells of the Pre-B-ALL.
- A negative staining of the plasmacytoma (only the remnants of normal B-cells should be demonstrated).
- A negative staining of all other cell types.

20 laboratories participated in this assessment. 60 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

**Table 1. Abs and assessment marks for CD19, run 35.**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff. <sup>1</sup>	Suff. OPS <sup>2</sup>
mAb clone <b>LE-CD19</b>	11	BioCare BioSite Dako Serotec	5	1	5	0	55 %	75 %
mAb clone <b>BT51E</b>	1	Novocastra/Leica	0	0	1	0	-	-
Not specified	2		1	0	1	0	-	-
<b>Ready-To-Use Abs:</b>								
mAb clone <b>LE-CD19, IR656</b>	4	Dako	3	1	0	0	100 %	100 %
mAb clone <b>BT51E, PA0843</b>	1	Novocastra/Leica	1	0	0	0	-	-
mAb clone <b>MRQ-36, 119M-17</b>	1	Cell Marque	0	0	0	1	-	-
<b>Total</b>	20		10	2	7	1	-	
<b>Proportion</b>			50 %	10 %	35 %	5 %	60 %	-

1) Proportion of sufficient stains (optimal or good),

2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

**Concentrated Abs**

mAb clone **LE-CD19**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either TRS pH 9 (2/3)\*, TRS pH 9 (3-in-1,Dako) (1/2), Cell Conditioning 1 (1/1) or Bond Epitope Retrieval Solution 2 (1/1). The mAb was diluted in the range of 1:25–1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 out of 11 (55 %) laboratories produced a sufficient staining (optimal or good).

\* (number of optimal results/number of laboratories using this buffer)

### Ready-To-Use Abs

mAb clone **LE-CD19 (IR656 Dako)**: The protocols giving an optimal result were all based on HIER using TRS pH 9 (3-in-1, Dako), an incubation time of 20 min of the primary Ab and a 2-step polymer system, EnVision (Dako K8000) as the detection system. Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **BT51E (PA0843, Leica/Novocastra)**: The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 1, and an incubation time of 20 min in the primary Ab and a 3-step polymer system, Bond Polymer Refine (Novocastra/Leica) as the detection system.

The most frequent cause of insufficient stains was:

- Too high concentration of the primary antibody.
- Too low concentration of the primary antibody.

The prevalent feature of the insufficient results was a false positive staining of normal T-cells seen with some LE-CD19 products (Fig. 1a). 15 laboratories were using clone LE-CD19. 11 of these were using Dako products (RTU or concentrate) on various platforms (Autostainer, Bond, IntelliPATH and BenchMark Ultra) and none of these showed false positive T-cell staining. The remaining 4 labs using clone LE-CD19 from Serotec, Biocare and BioSite all showed false positive T-cell staining. The reason for that is unclear. Common for these three products are a high antibody concentration. The Ab. concentration is app. 10 times higher than in the Dako concentrate. This might be part of the explanation to this pattern and underlining the importance of a carefully calibration of the LE-CD19 clone with special attention on the normal T-cells. Though the numbers are small the RTU Abs in general did perform well in this assessment, with Dako LE-CD19 RTU achieving sufficient marks in 4 out of 4 cases (100%). One lab used Leica/Novocastras BT51E RTU and achieved optimal mark. Among the RTU Abs only clone MRQ-36 from Cell Marque failed (1 case).

### Conclusion

The mAb clone LE-CD19 and BT51E are useful for the demonstration of CD19. HIER is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated with special attention to normal T-cells to be negative. Tonsil and appendix are both appropriate controls: The mantle zone B-cells, the germinal centre B-cells and the follicular dendritic cells must show a strong reaction. Weakly positive normal plasma can be seen. No other cells should stain.

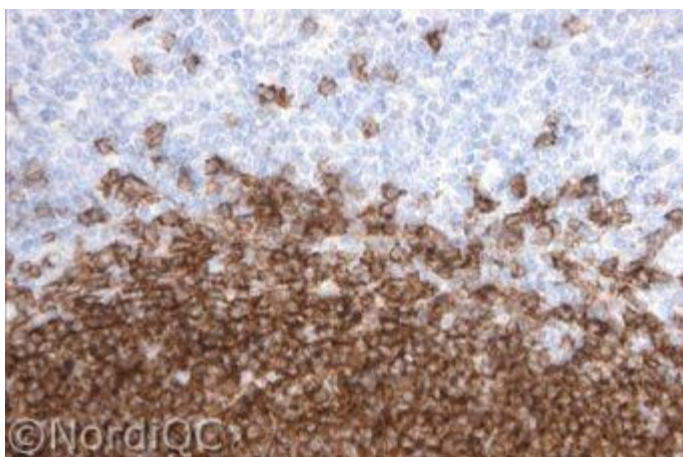


Fig. 1a  
Normal tonsil showing an optimal staining for CD19 using the mAb clone LE-CD19 from Dako, diluted 1:50, on the Autostainer platform. HIER was performed using TRS pH 9 (3-in-1) (Dako). A strong and distinct membranous staining reaction is seen in virtually all B-cells. T-cells are negative.

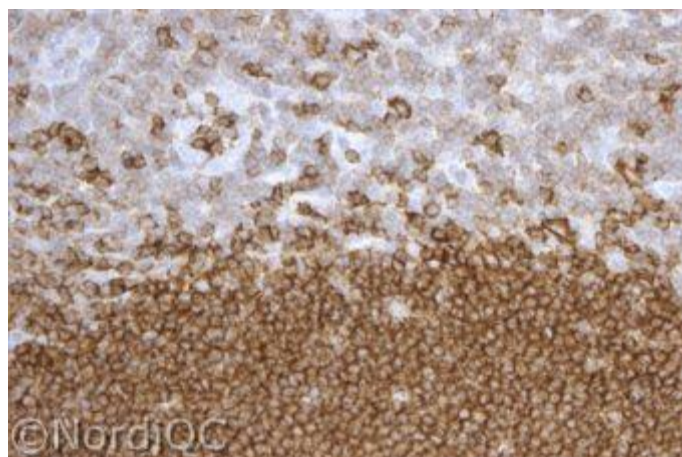
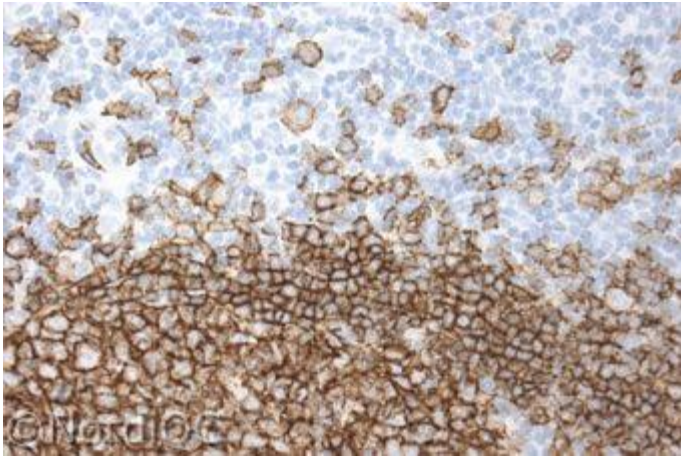
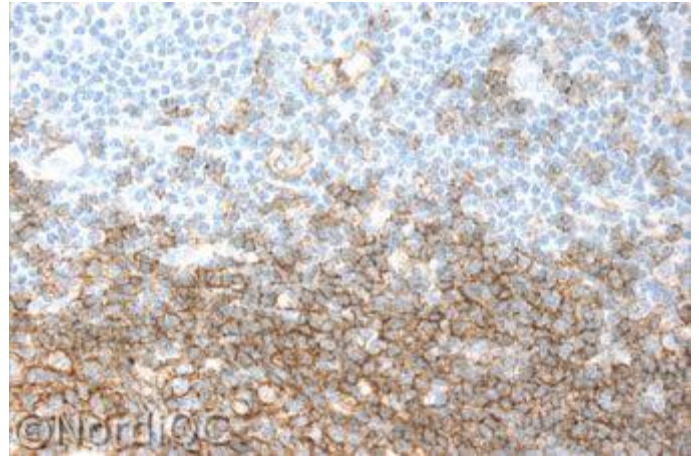


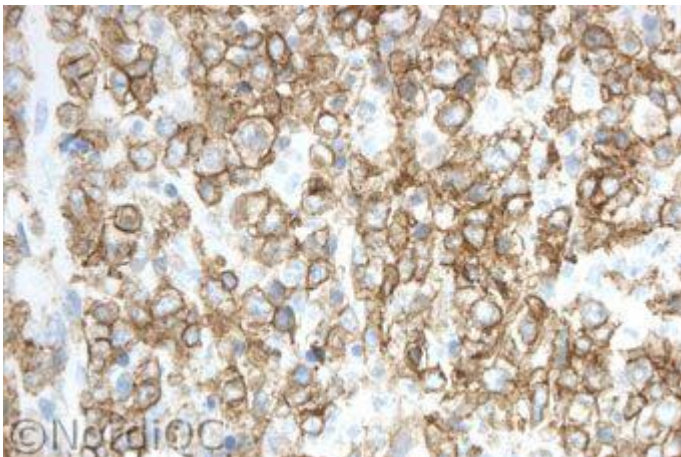
Fig. 1b  
Normal tonsil showing an insufficient staining for CD19 using the mAb clone LE-CD19 from Serotec, diluted 1:500, on the Autostainer platform. HIER was performed using Citrate pH 6. In addition to a moderate to strong staining reaction in the normal B-cells (albeit weaker than that seen in Fig 1a), the majority of T-cells shows a false positive staining reaction.



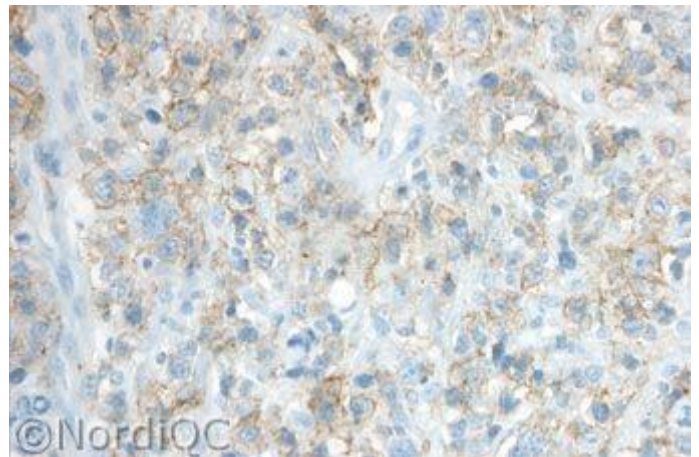
**Fig. 2a**  
Lymphatic tissue in the appendix showing an optimal staining for CD19 using the mAb clone BT51E (RTU) on the BOND-III platform. HIER was performed using Bond Epitope Retrieval Solution 1. A strong and very distinct membranous staining is seen in virtually all B-cells, while the T-cells are negative.



**Fig. 2b**  
Lymphatic tissue in the appendix showing an insufficient staining for CD19 using the mAb clone BT51E, diluted 1:30, on the BenchMark platform. HIER was performed using Cell Conditioning 1. Only a weak to moderate staining is seen in the majority of B-cells. T-cells are negative. Also compare with Fig. 3b, same protocol.



**Fig. 3a.** Optimal staining reaction for CD19 of the DLBCL. Same protocol used as in Fig. 2a based on the mAb clone BT51E. A moderate to strong membranous staining reaction is seen in virtually all the neoplastic cells.



**Fig. 3b.** Insufficient staining reaction for CD19 of the DLBCL using same protocol as in Fig. 2b. Only a weak staining is seen in scattered neoplastic cells. The majority of the tumour cells are negative. Compare with the optimal protocol in Fig. 3a, same field.

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