

The slide to be stained for CD23 comprised:

1. Tonsil fixed 24 h, 2. Tonsil fixed 72 h, 3-4. B-Chronic lymphatic leukaemia (B-CLL), 5. Mantle cell lymphoma (MCL).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD23 staining as optimal included:

- A strong, distinct membranous staining of the activated B-cells in the mantle zone of the germinal centres in the tonsils.
- A strong, distinct staining of the follicular dendritic cells in the germinal centres.
- An at least moderate, distinct membranous staining of the majority of the neoplastic cells in the two B-CLLs.
- No staining of the MCL.



114 laboratories submitted stains. At the assessment 21 achieved optimal marks (18 %), 43 good (38 %), 35 borderline (31 %) and 15 poor marks (13 %).

The following Abs were used:

mAb clone **1B12** (Novocastra/Leica, n=68; Ventana, n=9; Monosan, n=6; NeoMarkers/Thermo, n=4; Cell Marque, n=1; VECTOR, n=1; bio SB, n=1)

mAb clone **MHM6** (Dako, n=6)

rmAb clone **SP23** (NeoMarkers/Thermo, n=15; Dako, n=3)

Optimal staining for CD23 in this assessment was obtained with the mAb clone **1B12** (10 out of 90; 11%) and the rmAb clone **SP23** (11 out of 18; 61%).

All optimal protocols, independent of the Ab were based on heat induced epitope retrieval (HIER) using following buffers and protocol settings:

1B12: Tris-EDTA/EGTA pH 9.0 (5/43)*, Bond Epitope Retrieval Solution 2 (Bond, Leica) (3/6), EDTA/EGTA pH 8 (1/7), or Target Retrieval Solution pH 6.1 (Dako) (1/2). The mAb was diluted in the range of 1:20 – 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 38 out of 62 (61 %) laboratories produced a sufficient staining (optimal or good). If a 3-step polymer based detection system as Bond Refine, Leica Microsystems, or Powervision+, Immunovision was used in combination with the above mentioned protocol settings 13/14 laboratories obtained a sufficient result (of which 7 were optimal), indicating the clone 1B12 requires a very sensitive protocol for optimal performance.

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

SP23: Target Retrieval Buffer pH 9, (Dako) (3/6), Tris-EDTA/EGTA pH 9.0 (4/5), Citrate pH 6.0 (2/3) or Citrate pH 7.0 (1/1). The rmAb was diluted in the range of 1:20 – 1:100 depending on the total sensitivity of the protocol employed or as a Ready-To-Use Ab.

Using these protocol settings 15 out of 15 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:

- Less successful primary Ab
- Less successful RTU Ab clone 1B12 (8/10 insufficient)
- Too low concentration of the primary Ab
- HIER in non-alkaline buffer (typically Citrate pH 6.0)
- Use of low sensitive detection systems.

In this assessment and in concordance with the previous runs virtually all laboratories were able to demonstrate CD23 in the follicular dendritic network of the germinal centres, whereas the prevalent feature of an insufficient staining was a too weak or false negative staining of the neoplastic B-cells in the two B-CLLs. In general, CD23 is only weakly expressed in B-CLL and a highly sensitive protocol is required to demonstrate CD23 properly.

Normal tonsil was found to be a reliable quality indicator for the immunohistochemical demonstration of CD23, as the sufficient results all showed a moderate to strong membranous reaction of the activated mantle zone B-cells. If these B-cells were negative or only weakly demonstrated, the B-CLLs also were negative or only showed a dubious reaction.

Clone MHM6 and the RTU format of the clone 1B12 gave an acceptable reaction with the follicular dendritic cells, but in 5 out of 6 and 8 out of 10 protocols respectively, the staining reaction was insufficient in the B-cell lymphomas. Comparing the Abs used in the last 2 assessments for CD23 listed in table 1, these data indicates that the rmAb clone SP23 seems to be the most robust Ab for CD23.

Table 1. **Performance of five clones/formats in the last two runs.**

	Run 19 & 24 All protocol settings			Run 19 & 24 Optimal protocol settings*		
	Protocols	Sufficient	Optimal	Protocols	Sufficient	Optimal
mAb clone 1B12 conc.	139	84 (60%)	31 (22%)	128	84 (66%)	31 (24%)
mAb clone 1B12 RTU	19	3 (16%)	0 (0%)	19	3 (16%)	0 (0%)
mAb clone MHM6	16	1 (6%)	0 (0%)	16	1 (6%)	0 (0%)
rmAb SP23 conc.	23	20 (96%)	12 (52%)	21	20 (95%)	12 (57%)
rmAb SP23 RTU	3	3 (100%)	3 (100%)	3	3 (100%)	3 (100%)

*Appropriate HIER and dilution of the antibody (listed for each marker in the 2 assessments).

This was the third assessment of CD23. As seen in Table 2, the overall performance for CD23 has not improved. Focusing on 54 laboratories participating in all the 3 runs for CD23 an almost identical proportion of sufficient results is seen in this group as the pass rate was 78 % in run 8 and 59 % in this run 24. The relatively low proportion of sufficient results and lack of improvement of the pass rate in the 3 runs seems primarily related to the widely use of less successful Abs such as the clone MHM6 and clone 1B12 in RTU format. Moreover it became clear from the current assessment that clone 1B12 in concentrated format is difficult to optimize unless used with a three layer polymer conjugate system.

Table 2. **Proportion of sufficient results in three runs.**

	Run 9 2003	Run 19 2007	Run 24 2008
Participants, n=	59	88	114
Sufficient results	76%	54%	56%

The impact of the tailored recommendations was found be inferior to that in other assessments. 26 laboratories participating in run 19 obtained an insufficient mark and submitted a new staining in the current run. 18 laboratories followed the recommendations and 9 (50%) improved their mark to sufficient. 8 laboratories did not follow the recommendations and 2 improved their mark (25%). 6 laboratories changed their entire IHC system, and 3 of these improved to a sufficient mark. The most common recommendations given were to increase the Ab concentration for the clone 1B12 and to optimize HIER (prolong or use an alkaline buffer). For users of the clone 1B12 NordiQC recommend to use a 3-step polymer based detection system, alternatively to change to the rmAb clone SP23.

Conclusion

The rmAb clone **SP23** seems to be the most robust marker for the detection of CD23 in B-cell lymphomas. HIER is mandatory for optimal performance. The mAb clone **1B12** was less successful and should be used with HIER in an alkaline buffer and a 3-step polymer based detection system.

Tonsil is the recommended control: The activated mantle zone B-cells (10-50% of the cells) must show a distinct membranous reaction.

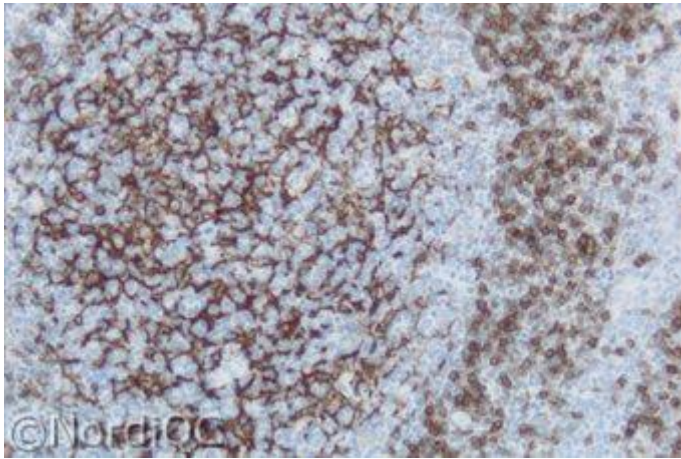


Fig. 1a
Optimal CD23 staining of a secondary follicle in the tonsil using the mAb clone 1B12 diluted **1:100**, HIER in an alkaline buffer and visualized with a **3-step polymer** based detection system. Both the follicular dendritic cells and the activated mantle zone B-cells show a strong and distinct staining.

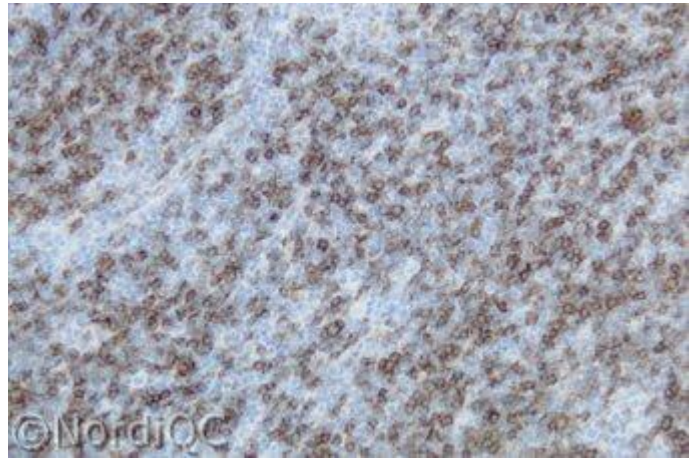


Fig. 1b
Optimal CD23 staining of the B-CLL no 3 in the assessment using same protocol as in Fig. 1a. The majority of the neoplastic cells show a strong and distinct membranous staining.

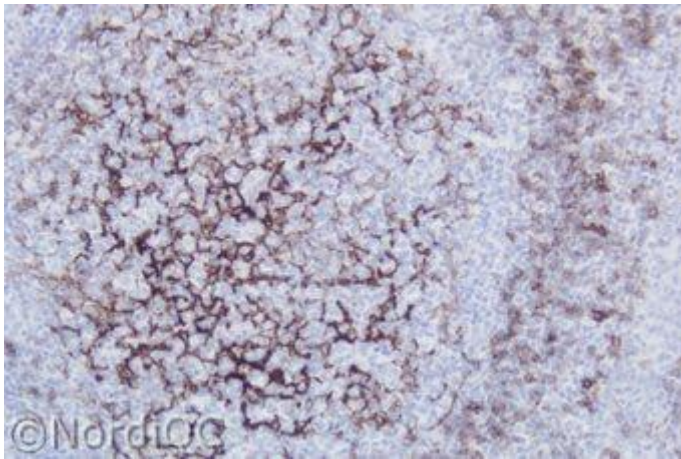


Fig. 2a
CD23 staining of a secondary follicle in the tonsil (same field as in Fig. 1a) using the mAb clone 1B12 diluted **1:50**, HIER in an alkaline buffer and visualized with a **2-step polymer** based detection system. The follicular dendritic cells and the activated mantle zone B-cells are demonstrated, but the reaction is slightly diffuse and with a reduced intensity. The staining was assessed as good.

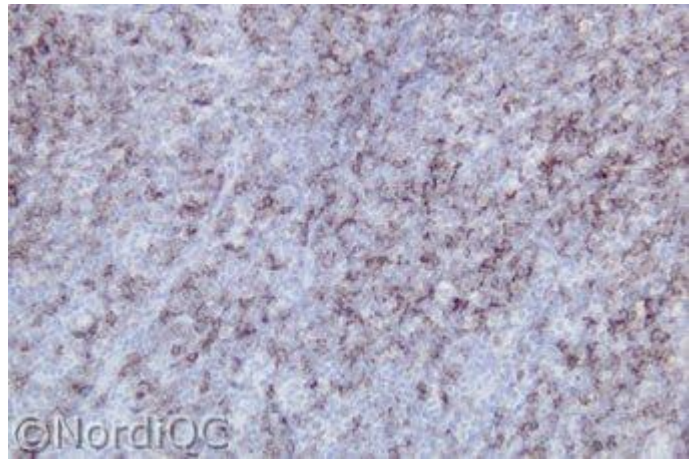


Fig. 2b
CD23 staining of the B-CLL no 3 in the assessment using same protocol as in Fig. 2a. (same field as in Fig. 1b). The majority of the neoplastic cells is demonstrated but show a diffuse incomplete membranous reaction.

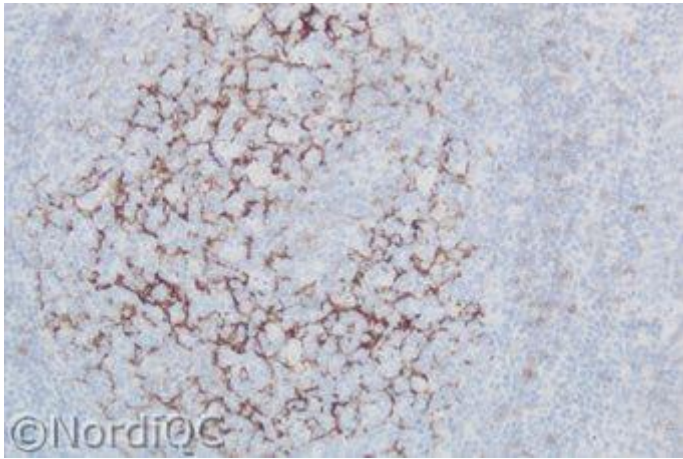


Fig. 3a
Insufficient CD23 staining of a secondary follicle in the tonsil (same field as in Fig. 1a). Only the follicular dendritic cells show a distinct staining, while the activated mantle zone B-cells are negative. The protocol was based on the mAb clone 1B12 as RTU, HIER in an alkaline buffer and a 2-step polymer based detection system.

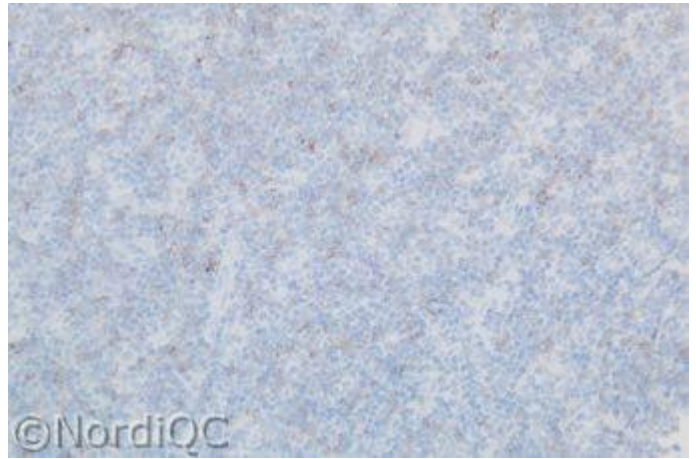


Fig. 3b
Insufficient CD23 staining of the B-CLL no 3 in the assessment using same protocol as in Fig. 3a. (same field as in Fig. 1b). The neoplastic cells are virtually negative.

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