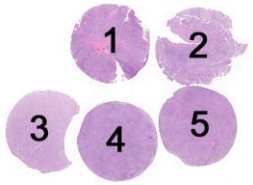
Assessment Run 34 2012
CD23

Material
The slide to be stained for CD23 comprised:

1. Tonsil, fixed 24h., 2. Tonsil, fixed 48h., 3. Mantle cell lymphoma, 4. & 5. B-CLL



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD23 staining as optimal included:

- An at least weak to moderate and 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 in the tonsils.
- An at least weak to moderate, distinct membranous staining of the majority of the neoplastic cells in the two B-CLLs.
- No staining of the neoplastic cells of the mantle cell lymphoma.

181 laboratories participated in this assessment. 73 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD23, run 34**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1B12**	61 6 5 1 1	Leica/Novocastra Thermo/NeoMarkers Monosan Biocare Cell Marque	21	28	24	1	66 %	86 %
mAb clone **DAK-CD23**	1	Dako	1	0	0	0	-	-
mAb clone **MHM6***	8	Dako	0	1	5	2	12 %	-
rmAb clone **EP73**	1	Epitomics	0	1	0	0	-	-
rmAb **SP23**	19 8 2 1 1	Thermo/NeoMarkers Dako* Spring Bioscience DBS Master Diagnostics	16	9	6	0	81 %	85 %
Ready-To-Use Abs:								
mAb clone **1B12 PA0169**	6	Leica	5	1	0	0	100 %	100 %
mAb clone **1B12 PM100**	1	Biocare	0	0	1	0	-	-
mAb clone **1B12 MONX10379**	1	Monosan	0	0	1	0	-	-
mAb clone **1B12 MS-729-R7**	1	Thermo/NeoMarkers	0	0	1	0	-	-
mAb clone **DAK-CD23 IS/IR781**	3	Dako	2	0	1	0	-	-
rmAb clone **SP23 790-4408**	29	Ventana	13	13	3	0	90 %	95 %
rmAb clone **SP23 IR800***	22	Dako	11	9	1	1	91 %	100 %

rmAb clone **SP23 123R-17**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **SP23 760-2616***	1	Ventana/Cell Marque	0	1	0	0	-	-
rmAb clone **SP23 RMA-0504**	1	Maixin	0	0	0	1	-	-
Total	181		69	63	44	5	-	
Proportion			38 %	35 %	24 %	3 %	73 %	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below
* Product has been discontinued by the vendor

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **1B12**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Bond Epitope Retrieval Solution 2 (BERS 2, Bond, Leica) (10/13)*, Target Retrieval Solution (TRS, 3-in-1, Dako) pH 9 (5/13), TRS pH 9 (Dako) (1/7), Tris-EDTA/EGTA pH 9 (3/12) or Diva Decloaker pH 6.2 (Biocare) (2/3) as the retrieval buffer.
The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 37 out of 43 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **DAK-CD23**: The protocol giving an optimal result was based on HIER using TRS pH 9 (Dako) as the retrieval buffer. The mAb was diluted 1:200.

rmAb clone **SP23**: The protocols giving an optimal result were all based on HIER using either TRS pH 9 (3-in-1, Dako) (4/6), TRS pH 9 (Dako) (2/5), Cell Conditioning 1 (CC1, BenchMark, Ventana) (4/10), BERS 2 (Bond, Leica) (2/2), Tris-EDTA/EGTA pH 9 (3/5) or Citrate pH 6.7 (1/1) as the retrieval buffer.
The mAb was typically diluted in the range of 1:40-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 23 out of 27 (85 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
mAb clone **1B12** (product.no. PA0169, Leica/Novocastra): The protocols giving an optimal result were all based on HIER using BERS 2 (Bond, Leica), an incubation time of 10-25 min in the primary Ab and Bond Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings 6 out of 6 laboratories (100 %) produced a sufficient staining.

mAb clone **DAK-CD23** (prod. no. IS/IR781, Dako): The protocols giving an optimal result were both based on HIER in PT-Link (heating time for 20 min at 97°C) using TRS low pH 6.1 (Dako) as HIER buffer, an incubation time of 20 min in the primary Ab and EnVision Flex+ (K8002) as the detection system. Using these protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining.

rmAb clone **SP23** (prod. no. 790-4408, Ventana): The protocols giving an optimal result were based on HIER in CC1 (BenchMark, Ventana) mild, standard or extended, an incubation time of 8-44 min in the primary Ab and iView (760-091) UltraView (760-500) with or without amplification kit or OptiView as the detection system.
Using these protocol settings 40 out of 42 (95 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody
- Use of detection systems with a too low sensitivity
- Less successful primary Ab
- Less successful performance of the mAb clone 1B12 on the BenchMark, Ventana

In this assessment and in concordance with the previous NordiQC assessments for CD23 the prevalent feature of an insufficient staining was a too weak or false negative reaction of the cells expected to be demonstrated. A too weak or false negative staining reaction was seen in 96 % of the insufficient results (47 out of 49) and in the remaining 4 % an impaired morphology was seen.

Virtually all laboratories were able to demonstrate CD23 in the follicular dendritic cells in the germinal centres of the tonsils, whereas the prevalent feature of an insufficient staining was characterized by a too weak or false negative staining of both the activated mantle zone B-cells and the neoplastic B-cells of the two B-CLLs.

The tonsil was found to be a reliable control for CD23 provided that the activated mantle zone B-cells showed an at least weak to moderate but distinct continuous membranous staining reaction. If these cells were negative or only weakly demonstrated giving a patchy membranous staining, the neoplastic cells in the two B-CLL lymphomas and in particular the B-CLL tissue core no. 5 were negative or only showed an equivocal staining reaction.

A too low concentration of the primary and/or the use of detection systems with a low sensitivity were the main reasons for the insufficient results of the mAb clone 1B12 and the rmAb clone SP23 when used as a concentrate.
The pass rate and proportion of optimal results in particular for the mAb clone 1B12 was influenced by the sensitivity of the detection system used. If the mAb clone 1B12 was used in the range of 1:20-100 with a 2-step polymer or multimer based detection system as e.g., EnVision Flex, Dako or UltraView, Ventana, 18 out of 26 laboratories obtained a sufficient staining result (69%) out of which 3 (12%) were assessed as optimal. If a more sensitive 3-step polymer or multimer based detection system as e.g., EnVision Flex+, Bond Refine (Leica) or UltraView + amplification was used, 20 out of 23 laboratories produced a sufficient staining result (87%) of which 15 (65%) were assessed as optimal.
For the rmAb clone SP23 used in the range of 1:20 - 100 with a 2-step polymer or multimer based detection system a pass rate of 74% was seen, out of which 57% were optimal and by a 3-step polymer or multimer based detection system a pass rate of 100% was seen, out of which 80% were optimal.
A significant difference in the overall performance for the most widely used mAb clone 1B12 for CD23 as a concentrate was also related to the IHC platform applied. Only 6 out of 19 (32 %) protocols performed on the fully automated platform BenchMark XT or Ultra, Ventana were assessed as sufficient, none were optimal. In contrast, 14 out of 15 (93 %) protocols performed on a similar fully automated platform Bond-max or Bond III were assessed as sufficient, out of which 10 (67 %) were optimal.
For the rmAb clone SP23 7 out of 10 (70%) protocols performed on the BenchMark platform was assessed as sufficient, out of which 4 (40%) were optimal. As similar protocol settings were applied for the mAb clone 1B12 and the rmAb clone SP23 on the BenchMark, this clearly indicates that the rmAb clone SP23, should be the preferred choice for this IHC platform.

7 out of 8 protocols based on the mAb clone MHM6 were assessed as insufficient irrespective of the protocol settings being identical to the settings giving an optimal staining for both the mAb clone 1B12 and the rmAb clone SP23. The mAb clone MHM6 typically gave a satisfactory staining result in the follicular dendritic cells with a high antigen expression, but gave a too weak or completely false negative staining reaction in cells with a reduced CD23 expression as both the normal activated mantle zone B-cells and the neoplastic cells of the B-CLL lymphomas. In total 24 protocols/slides based on the mAb clone MHM6 have been submitted to NordiQC in the last 3 runs for CD23 (runs 19, 24 and 34) and only 8 % (2 protocols) has provided a sufficient staining result (assessed as good) in these assessments. These data clearly supports that this mAb clone MHM6 can not be recommended as marker for CD23 for diagnostic use and should be replaced by another clone. This has also been effectuated by Dako having discontinued the product and replaced this by the mAb clone DAK-CD23.

This was the 4th assessment of CD23 in NordiQC (Table 2), and a major increase in the pass rate is seen from run 24, 2008 to this actual run 34, 2012.

Table 2. **Proportion of sufficient results for CD23 in the four NordiQC runs performed**

	Run 8 2003	**Run 19 2007**	**Run 24 2008**	**Run 34 2012**
Participants, n=	59	88	114	181
Sufficient results	76 %	54 %	56 %	73 %

The significant improvement of the pass rate for CD23 most likely is highly influenced by the expanding use of sensitive 3-step polymer or multimer based detection systems providing a more robust assay. It is also related to the improved Ready-To-Use (RTU) systems for CD23 from the main providers as Leica, Ventana and Dako. The RTU systems in this assessment showed a pass-rate of 95%-100% thus being superior to the pass-rates for the in-house validated protocols for CD23.
In this assessment for CD23 many new laboratories participated for the first time and for these a slightly lower pass rate was observed compared to the laboratories also participating in the previous run 24, 2008:

For the laboratories participating for the first time the pass rate was 68 % (56 out of 82), whereas the pass rate was 77 % (76 out of 99) for the laboratories participating in both runs.

Conclusion

The mAb clones 1B12 and DAK-CD23 and the rmAb clone SP23 are all recommendable Abs for the demonstration of CD23. Both the concentrated formats and the Ready-To-Use formats of these clones gave a high proportion of sufficient results.

Efficient HIER in combination with a highly sensitive 3-step polymer or multimer based detection system was mandatory for an optimal performance. The protocol must be carefully calibrated in order to detect the low antigen expression of CD23 in the neoplastic cells of B-CLL.

Normal tonsil is an appropriate control provided that at least a weak to moderate but distinct continuous membranous staining reaction is seen in the activated B-cells in the mantle zone of the secondary follicles in the tonsils.

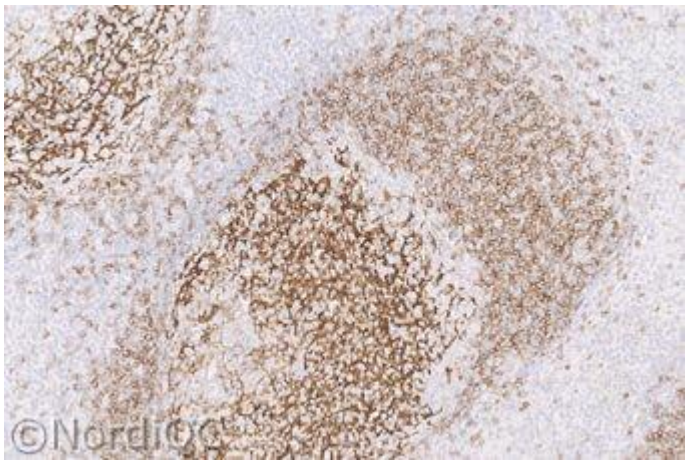


Fig. 1a
Optimal staining for CD23 of the tonsil no. 1 fixed 24 h. in NBF using the rmAb clone SP23 optimally calibrated, HIER in an alkaline buffer and a 3-step multimer conjugate.
The activated mantle zone B-cells show a moderate staining reaction, which even at low magnification (x100) can be identified. The follicular dendritic cells show an intense staining reaction.
Also compare with Figs. 2a – 4a, same protocol.

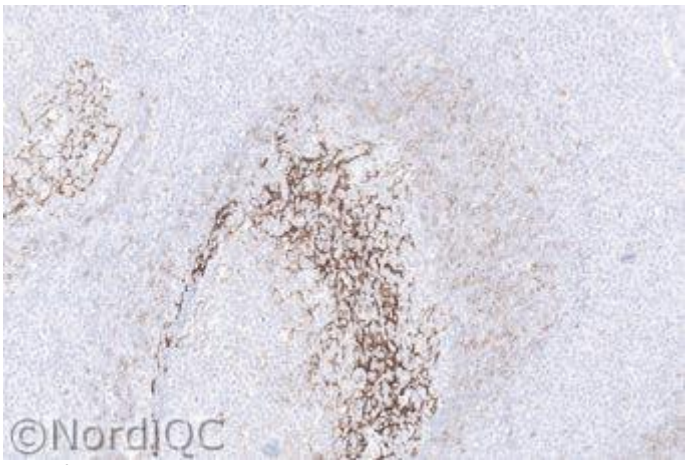


Fig. 1b
Insufficient staining for CD23 of the tonsil no. 1 fixed 24 h. in NBF using the mAb clone 1B12 by a protocol with a too low sensitivity (too low. conc. of the primary Ab and a 2-step multimer conjugate) - same field as in Fig. 1a.
Only the follicular dendritic cells show a distinct staining reaction, whereas the activated mantle zone B-cells show an equivocal staining reaction.
Also compare with Figs. 2b - 4b, same protocol.

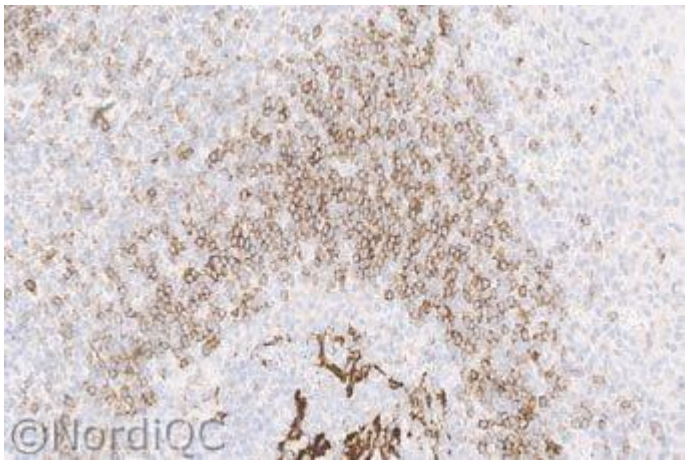


Fig. 2a
Optimal staining for CD23 of the tonsil no. 2 fixed 48 h. in NBF using same protocol as in Fig. 1a – high magnification x200.
The activated mantle zone B-cells show a distinct moderate staining reaction with a continuous membranous appearance.

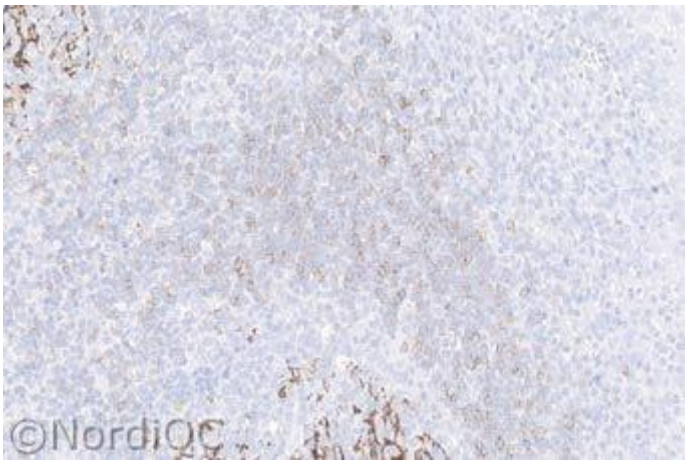


Fig. 2b
Insufficient staining for CD23 of the tonsil no. 2 fixed 48 h. using same protocol as in Fig. 1b - same field as in Fig. 2a – high magnification x200
Only the follicular dendritic cells show a distinct staining reaction, while the activated mantle zone B-cells virtually are false negative and only a patchy staining is seen.

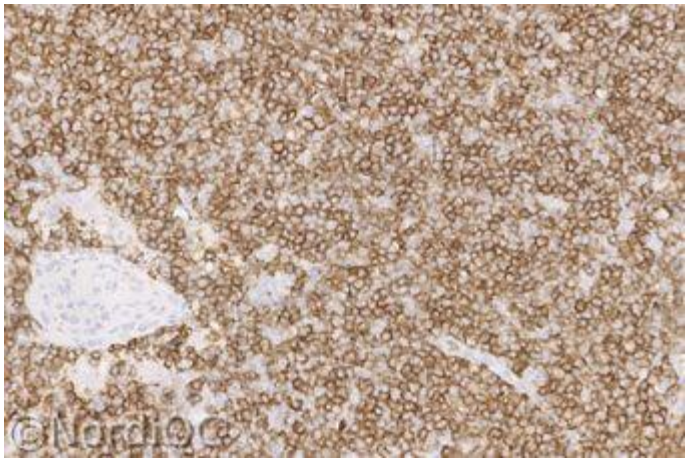


Fig. 3a
Optimal staining for CD23 of the B-CLL no. 4 using same protocol as in Figs. 1a - 2a. Virtually all the neoplastic cells show a moderate to strong and distinct staining reaction. No background staining is seen.

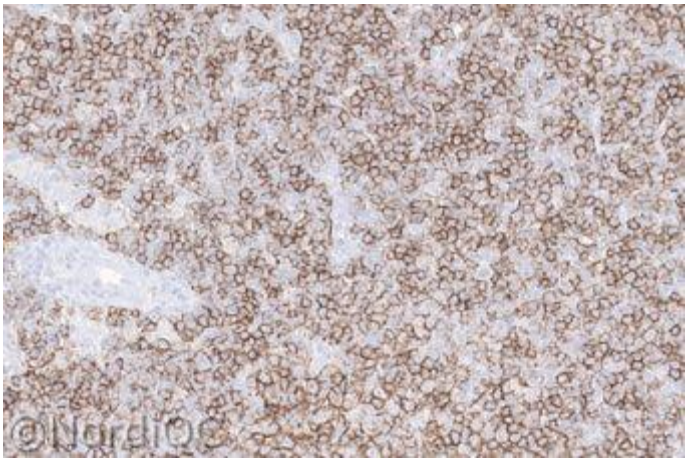


Fig. 3b
Staining for CD23 of the B-CLL no. 4 using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The majority of the neoplastic cells are demonstrated; however a less distinct membranous staining reaction is seen. Also compare with Fig. 4b.

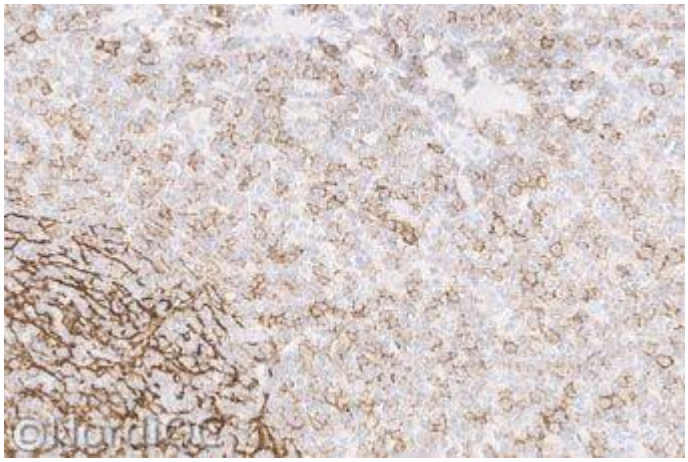


Fig. 4a
Optimal staining for CD23 of the B-CLL no. 5 using same protocol as in Figs. 1a - 3a.
The majority of the neoplastic cells show a weak to moderate and distinct staining reaction, while remnants of the follicular dendritic network show a strong staining reaction.

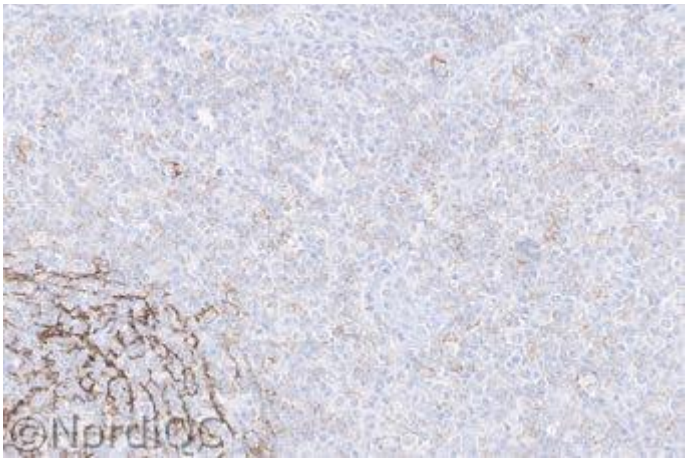


Fig. 4b
Insufficient staining for CD23 of the B-CLL using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The neoplastic cells are virtually negative and only the follicular dendritic network is demonstrated..

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