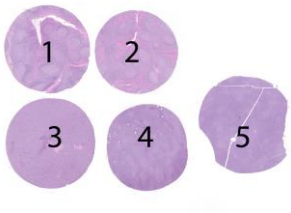
Assessment Run 50 2017
CD23

Material
The slide to be stained for CD23 comprised:



1-2. Tonsil, 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, distinct membranous staining reaction of the majority of 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 moderate to strong, distinct membranous staining of the majority of neoplastic cells in the two B-CLLs.
- No staining of neoplastic cells in the mantle cell lymphoma. Only remnants of the follicular dendritic cell meshwork should be stained.
- No staining of T-cells or squamous epithelial cells in the tonsils.

Participation

Number of laboratories registered for CD23, run 50	283
Number of laboratories returning slides	270 (95%)

Results
270 laboratories participated in this assessment. 241 (89%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Insufficient HIER (too low temperature and/or too short heating time)
- Less sensitive detection systems
- Less successful performance of the mAb clone 1B12 on the BenchMark, Ventana
- Unexplained technical issues

Performance history
This was the fifth NordiQC assessment of CD23. The overall pass rate was significantly higher compared with all previous runs for CD23 (see Table 2).

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

	Run 8 2003	Run 19 2007	Run 24 2008	Run 34 2012	Run 50 2017
Participants, n=	59	88	114	181	270
Sufficient results	76%	54%	56%	73%	89%

Conclusion
The mAb clones **1B12, DAK-CD23, BS20** and the rmAb clone **SP23** could all be used to obtain optimal demonstration of CD23. Irrespective of the primary Ab applied, efficient HIER and use of a sensitive 3-step polymer/multimer detection system were the most important prerequisites for an optimal staining result. The RTU systems based on the mentioned clones (IR/GA781, PA0169 and 790-4408 from Agilent/Dako, Leica/Novocastra and Roche/Ventana, respectively) all provided a high proportion of sufficient results and was superior in performance compared to the laboratory developed assays based on the same clones. Tonsil is recommended as positive and negative tissue control: The follicular dendritic cells of germinal centres must show a strong staining reaction and the majority of activated B-cells in the mantle zone of the follicles must display an at least weak to moderate distinct, membranous staining reaction. No staining reaction must be seen in other cell types including squamous epithelial cells and T-cells in the interfollicular T-zones of the tonsil. Dispersed B-cell may be positive.

Table 1. **Antibodies and assessment marks for CD23, run 50**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1B12**	51 3 2 2 2	Leica/Novocastra Cell Marque Biocare Thermo F. Scientific Monosan	22	27	8	3	82%	87%
mAb clone **DAK-CD23**	12	Agilent/Dako	5	4	2	1	75%	100%
mAb clone **BS20**	1	Nordic Biosite	1	0	0	0	-	-
mAb clone **MRQ-57**	1		0	0	1	0	-	-
mAb clone **MHM6***	1	Agilent/Dako	1	0	0	0	-	-
rmAb clone **SP23**	25 3 3 1 1	Thermo S./ Neomarkers Spring Bioscience Cell Marque Immunologic Diagnostic Biosystems	20	9	4	0	88%	90%
Ready-To-Use antibodies								
mAb clone **1B12 PA0169**	9	Leica/Novocastra	8	0	1	0	89%	100%
mAb clone **1B12**[3] **PA0169**	3	Leica/Novocastra	0	2	1	0	-	-
mAb clone **1B12 123M-18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **1B12 PM100**	1	Biocare	0	1	0	0	-	-
mAb clone **1B12 PDM143**	1	Diagnostic Biosystems	0	0	1	0	-	-
mAb clone **DAK-CD23 IR781**	31	Agilent/Dako	24	5	1	1	94%	92%
mAb clone **DAK-CD23 IR781**[4]	7	Agilent/Dako	3	4	0	0	100%	-
mAb clone **DAK-CD23 GA781**	15	Agilent/Dako	14	1	0	0	100%	100%
mAb clone **DAK-CD23 GA781**[5]	1	Agilent/Dako	0	1	0	0	-	-
mAb clone **GR013 8262-C010**	1	Sakura	1	0	0	0	-	-
rmAb clone **SP23 790-4408**	78	Roche/Ventana	43	34	1	0	99%	99%
rmAb clone **SP23 123R-17/18**	5	Cell Marque	3	1	1	0	80%	100%
rmAb clone **SP23 MAD-00333QD**	3	Master Diagnostica	2	0	0	1	-	-
rmAb clone **SP23 M3231**	2	Spring Bioscience	0	2	0	0	-	-
rmAb clone **SP23 RMA-0504**	1	Maixin	0	1	0	0	-	-
rmAb clone **SP23 IR800***	1	Agilent/Dako	1	0	0	0	-	-
rmAb clone **EP75 123R-27/28**	1	Cell Marque	1	0	0	0	-	-
pAb **AR460-5/10R**	1	Biogenex	0	0	0	1	-	-
Total	270		149	92	22	7	-	
Proportion			55%	34%	8%	3%	89%	

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only, see below. 3) RTU system developed for the Leica/Novocastra full-automatic system (BOND III/MAX) but used by laboratories on e.g. a Ventana Benchmark Ultra (Roche/Ventana), 4) RTU system developed for the Agilent/Dako semi-automated systems (Autostainer) but used by laboratories on the Omnis (Agilent/Dako). 5) RTU used in a manual assay. *Product has been discontinued by the vendor.

Detailed analysis of CD23, Run 50
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **1B12**: Protocols with optimal results were based on heat induced epitope retrieval (HIER) using Bond Epitope Retrieval Solution 2 (BERS2, Leica) (8/14)*, Target Retrieval Solution (TRS, 3-in-1, Dako) pH 9 (6/11) or Cell Conditioning 1 (CC1, Ventana) (7/20) as retrieval buffer.
The mAb was typically diluted in the range of 1:10-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 33 of 38 (87%) laboratories produced a sufficient staining (optimal or good). One protocol with an optimal result was based on no pre-treatment at all.
* (number of optimal results/number of laboratories using this buffer)

mAb clone **DAK-CD23**: Protocols with optimal results were all based on HIER using TRS (3-in-1) pH 6.1 (Dako) (3/3) or BERS2 (Leica) (2/3) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 6 (100 %) laboratories produced a sufficient staining.

mAb clone **BS20**: One protocol with an optimal result was based on HIER using TRIS-EDTA/EGTA pH 9 as retrieval buffer. The mAb was diluted 1:200 and a 2-step polymer based detection system (Nordic Biosite) was used.

rmAb clone **SP23**: Protocols with optimal results were all based on HIER using CC1 (Ventana) (11/20), TRS (3-in-1) pH 9 (Dako) (1/2), BERS2 (Leica) (3/3), Tris-EDTA/EGTA pH 9 (2/5), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/1), TRS (3-in-1) pH 6.1 (1/1) or Citrate pH 6.7 (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 26 of 29 (90%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for CD23 for the most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	**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 **1B12**	4/6** (67%)	-	2/4	-	7/19 (37%)	-	8/10 (80%)	0/2
mAb clone **DAK-CD23**	0/3	3/3	-	-	0/1	-	2/3	-
rmAb clone **SP23**	1/1	-	0/1	1/1	10/17 (59%)	0/1	3/3	1/1

* 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 clone **DAK-CD23,** product no. **IR781,** Agilent/Dako Autostainer+ /Autostainer Link:
Protocols with optimal results were based on HIER using TRS (3-in-1) pH 9 or pH 6.1 (efficient heating time 10-20 min. at 95-99°C), 20-60 min. incubation of the primary Ab and EnVision Flex/Flex+ (K8000/K8002) as detection system. Using these protocol settings, 24 of 26 (92%) laboratories produced a sufficient result. One laboratory obtained an optimal result using the same protocol settings as above but without performing any pre-treatment at all.

mAb clone **DAK-CD23,** product no. **GA781,** Agilent/Dako Omnis:
Protocols with optimal results were based on HIER using TRS (3-in-1) pH 6.1 (efficient heating time 30 min. at 97°C), 10-30 min. incubation of the primary Ab and EnVision Flex (GV800/GV823) with or without mouse linker (GV821) as detection system. Using these protocol settings, 14 of 14 (100%) laboratories produced a sufficient result (all assessed as optimal).

mAb clone **1B12,** product no. **PA0169,** Leica/Novocastra BOND III/BOND MAX:
Protocols with optimal results were based on HIER using BERS2 or BERS1 (efficient heating time 10-20 min. at 95-100°C), 15-30 min. incubation of the primary Ab and BOND Refine (DS9800) as detection system. Using these protocol settings, 8 of 8 (100%) laboratories produced a sufficient result (all assessed as optimal).

mAb clone **GR013,** product no. **8262-C010**, Sakura Finetek, Genie:
One protocol with an optimal result was based on HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer (efficient heating time 60 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system.

rmAb clone **SP23,** product no. **790-4408,** Ventana Benchmark Ultra/XT:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-98 min. at 95-100°C) and 16-60 min. incubation of the primary Ab. UltraView (760-500) or OptiView (760-700) with or without amplification (760-080 or 860-099, respectively) were used as detection systems. Using these protocol settings, 66 of 67 (99%) laboratories produced a sufficient staining result.

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

Table 4. **Proportion of sufficient and optimal results for CD23 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb **IR781**	100% (7/7)	100% (7/7)	92% (22/24)	71% (17/24)
Dako Omnis pAb **GA781**	100% (7/7)	100% (7/7)	100% (4/4)	75% (3/4)
Leica BOND MAX/III pAb **PA0169**	100% (4/4)	100% (4/4)	80% (4/5)	80% (4/5)
VMS Ultra/XT pAb **790-4408**	100% (3/3)	0% (0/3)	99% (71/72)	59% (43/72)

* 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 >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In this assessment and in concordance with the previous NordiQC CD23 assessments, the prevalent features of an insufficient staining result were too weak or completely false negative staining reaction of cells expected to be demonstrated. This pattern was seen in all (29 of 29) of the insufficient results. The majority of participating laboratories were able to demonstrate CD23 in the follicular dendritic cell meshwork of the germinal centres in the tonsils, whereas demonstration of mantle zone B-cells and the neoplastic cells of the B-CLL, tissue core no. 4, was more challenging and only seen with appropriate protocol settings.

40% (108 of 270) of the laboratories used a concentrated Ab format within laboratory developed (LD) assays for the demonstration of CD23. The mAb clone 1B12 was the most widely used Ab and could be used to obtain optimal results as shown in Tables 1 and 3. Used within a LD assay, the mAb clone 1B12 gave an overall pass rate of 82% (49 of 60) of which 37% (22 of 60) were optimal.
HIER in an alkaline buffer, careful calibration of the primary Ab in combination with a sensitive 3-step detection system seem to be the most critical parameters for a sufficient result.
As noted in the previous runs for CD23, identification of a protocol within a LD assay for mAb clone 1B12 to provide optimal results can be challenging, especially on the Ventana Benchmark. On this IHC platform, efficient HIER in CC1 (average HIER time of 50 min., range 24-64 min.), high concentration of the primary Ab (1:10-1:20) and a 3 step multimer detection system (UltraView with amplification or OptiView) were the central requirements for optimal performance. Using these protocol settings, 100 % (7 of 7) produced a sufficient result of which 86% (6 of 7) were assessed optimal.
The requirements for optimal performance for mAb clone 1B12 were basically the same on all other platforms. Using the automatic platforms from Agilent/Dako (Autostainer and Omnis) and Leica (BOND III/MAX), optimal results were typically obtained by efficient HIER in alkaline buffer (TRS pH 9, Dako or BERS2, Leica - average HIER time of 24 min., range 20-30 min.), a high antibody concentration (average dilution factor of 1:42; range 1:20-1:60) and the use of a 3-step polymer detection system (Flex+, Dako or BOND Refine, Leica). Protocols with insufficient results were typically based on a reduced HIER (average HIER time of 19 min. - range 10-30 min.), lower titer of the primary Ab (average dilution factor of 1:155; range 1:25-1:400) and use of a 2-step polymer system (Flex, Dako).
In this assessment, protocols based on the mAb 1B12 as concentrate and performed within a LD assay on the BOND III/MAX staining systems (Leica), provided the highest number of optimal results (see Table 3).

Twelve laboratories used the mAb DAK-CD23 within a LD assay and 75% (9 of 12) produced a sufficient result of which 42% (5 of 12) were optimal. Use of a sensitive detection system was the most important parameter for optimal performance. Using HIER in high and low pH buffers, dilution range of the primary Ab between 1:25-1:150 and use of a 2-step polymer/multimer detection system, 50% (3 of 6) of the protocols provided a sufficient result of which none were optimal. In comparison, using the same conditions with a 3-step polymer/multimer detection system, 100% (6 of 6) of the protocols provided a sufficient result of which 83% (5 of 6) were optimal.

The LD assays based on the rmAb SP23 provided a high number of sufficient and optimal results (see Table 1 and 3). The majority (64%, 21 of 33) of protocols were applied on a Ventana Benchmark platform and the overall pass rate for this platform was 90% (19 of 21) of which 52% (11 of 21) were optimal. The rmAb SP23 seems to be a robust antibody and for the Ventana Benchmark it provided higher number of sufficient and optimal results compared to the mAb 1B12 within a LD assay. The overall pass rate for the mAb 1B12 on the Ventana Benchmark was 75% (18 of 24) and only 29% (7 of 24) were assessed as optimal.
Applying the rmAb SP23 within a LD assay on the Benchmark platform, using HIER in CC1 (efficient heating time 24-98 min. at 95-100°C), a dilution range of 1:20-1:100 of the primary Ab (16-60 min. incubation time) and UltraView with amplification or OptiView as the detection system, 100% (15 of 15) of the protocols provided a sufficient result of which 67% (10 of 15) were optimal. On non-Ventana platforms, the pass rate was 83% (10 of 12) and 75% (9 of 12) optimal. The protocol settings for optimal performance were typically based on HIER preferable in alkaline buffer and a dilution range of 1:40-1:100 of the primary Ab.

60% (162 of 270) of the laboratories used a Ready-To-Use (RTU) system for CD23.
In general, the RTU systems from the three major vendors (Agilent/Dako, Roche/Ventana and Leica/Novocastra) all gave a high proportion of sufficient and optimal results and was in this assessment superior to the LD assays based on the same clones. Both vendor and laboratory modified protocol settings could be used to produce sufficient results. It was observed that protocols performed according to recommendations provided by the respective vendors provided a pass rate of 100% (see Table 4).

The Ventana RTU system for the BenchMark IHC platform based on rmAb clone SP23 (790-4408) was used by 78 participants. An overall pass rate of 99% (77 of 78) was seen and 55% (43 of 78) were optimal. Optimal results could only be obtained by use of laboratory modified protocol settings using UltraView + amplification or OptiView as the detection system. If the protocols were performed according to the recommendations provided by Ventana (HIER in CC1 64 min., 16 min. incubation with primary Ab and UltraView), none of three submitted protocols provided optimal results – all were assessed as good. Using all protocol settings and UltraView as the detection system, the pass rate was 91% (30 of 33) but only 15% (5 of 33) were optimal. In comparison, using exactly the same conditions except but applying OptiView or UltraView with amplification, the pass rate was 98% (44 of 45) and 84% (38 of 45) were optimal. One protocol was assessed as insufficient due to technical issues. The reason for this could not by identified from the protocol as this was similar to protocols giving optimal marks.

The RTU systems IR/GA781 based on mAb clone DAK-CD23 (Dako) were used by 46 laboratories. An overall pass rate of 96% (44 of 46) was seen and 83% (38 of 46) were optimal. Following the vendor recommendations for these two RTU systems, 100% (14 of 14 protocols) were assessed as optimal (see table 4). The RTU system GA781 (Omnis) provided a pass rate of 100% (15 of 15) of which 93% (14 of 15) were optimal. It was the RTU system providing the highest proportion of optimal results. For optimal performance, the basic protocol settings (vendor recommended) is based on HIER in TRS pH 6.1 (30 min. at 97°C), incubation of the primary Ab for 25 min. and use of Flex+ (GV800/823 + GV821) as detection system. The single laboratory that received the assessment score good (see Table 3) used a reduced HIER time of 10 min.
The RTU systems IR/GA781 were used off-label (on other automatic platforms or manually) by 8 laboratories. All were assessed as sufficient (see Table 1).

The RTU system PA0169 based on mAb clone 1B12 (Leica) was used by 9 laboratories. An overall pass rate of 89% (8 of 9) was seen and 89% (8 of 9) were optimal. Following the vendor recommendations for this RTU system, 100% (4 of 4) were assessed as optimal (see Table 4). For optimal performance, the protocol settings were based on HIER in BERS2 (20 min. at 99-100°C), incubation of the primary Ab for 15-30 min. and the use of Bond refine (DS9800) as the detection system. One laboratory obtained an insufficient mark (see Table 3), although protocol settings was similar to protocols giving optimal results.

This was the fifth assessment of CD23 in NordiQC (Table 2). A pass rate of 89% was obtained, which is a significant improvement compared to 73% in run 34, 2012. The proportion of laboratories using a RTU

system has increased significantly, 60% in this assessment compared to 36 % in run 34. In this run, the overall performance of the RTU systems from the three major vendors was superior compared to the LD assays and following vendor recommendations all protocols were assessed as sufficient. The extended use of robust RTU systems in this assessment, accounted for the overall improvement of the pass rate. For all Abs applied, especially the RTU format based on the rmAb SP23 (790-4408, Ventana), use of a sensitive 3-step polymer/multimer system gave the highest number of optimal results, provided that efficient HIER and for the concentrated Abs, careful calibration of the titre, was performed.

Controls

Tonsil is recommended as positive and negative tissue control for CD23. The follicular dendritic cells of the germinal centres must be stained as strongly as possible without any staining reaction of squamous epithelial cells and T-cells in the interfollicular T-zones. In the mantle zone of the follicles, the majority of activated B-cells must show an at least weak to moderate and distinct continuous membranous staining reaction. If these cells were negative or only weakly demonstrated with a patchy membranous staining reaction, the neoplastic cells in the two B-CLL lymphomas and in particular the B-CLL tissue core no. 4 were negative or showed only an equivocal staining reaction.

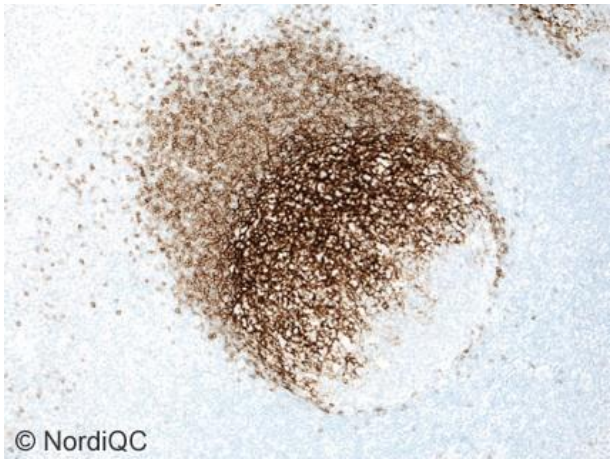


Fig. 1a (x100)
Optimal staining reaction for CD23 of the tonsil using the mAb clone 1B12 as concentrate, carefully calibrated (1:10), HIER in an alkaline buffer (CC1, Ventana) and a 3-step multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 3a. The majority of B-cells in the mantle zone show a moderate but distinct membranous staining reaction. The follicular dendritic cells of the germinal centres display a strong staining reaction - compare with Fig. 1b.

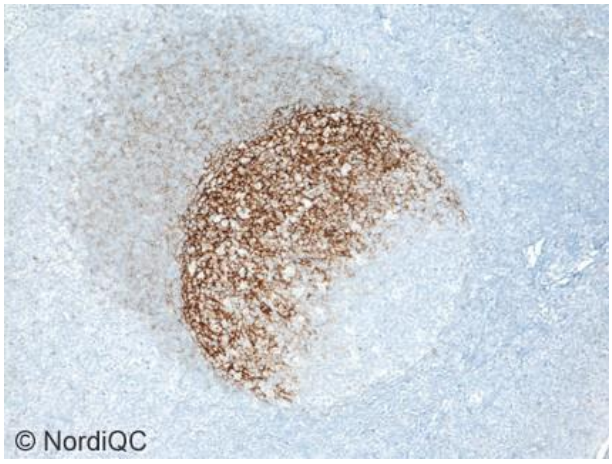


Fig. 1b (x100)
Insufficient staining reaction for CD23 of the tonsil using the mAb clone 1B12 as concentrate (too diluted, 1:50), HIER in CC1 and with a detection system giving a too low sensitivity (UltraView, Ventana) - same protocol used in Figs. 2b - 3b. The intensity of the staining reaction is significantly reduced and the majority of B-cells in the mantle zone show an equivocal staining reaction - compared with Fig. 1a (same field).

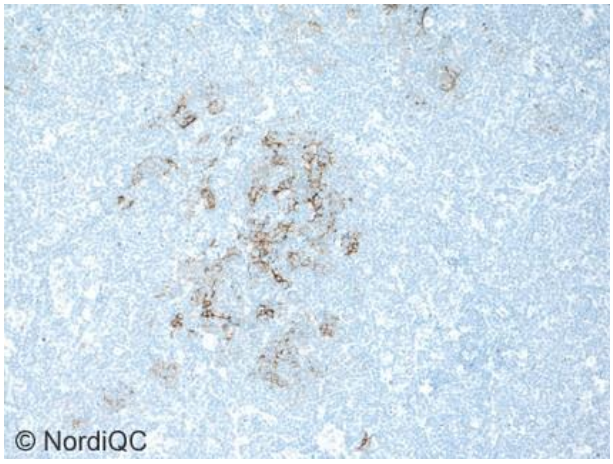


Fig. 2a (x100)
Optimal staining reaction for CD23 of the mantle cell lymphoma using the same protocol as in Fig. 1a. The neoplastic cells are negative and only remnants of the follicular dendritic cell meshwork show a strong staining reaction - compare with Fig. 2b.

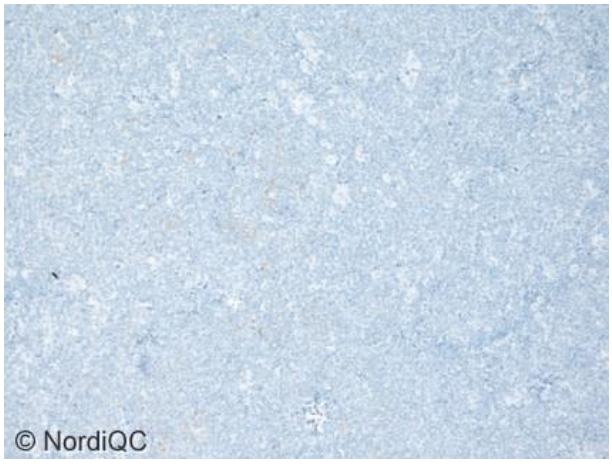


Fig. 2b (x100)
Insufficient staining reaction for CD23 of the mantle cell lymphoma using same protocol as in Fig. 1b - same field as in Fig. 2a. The intensity of the staining reaction is significantly reduced. The follicular dendritic cell meshwork is barely visible.

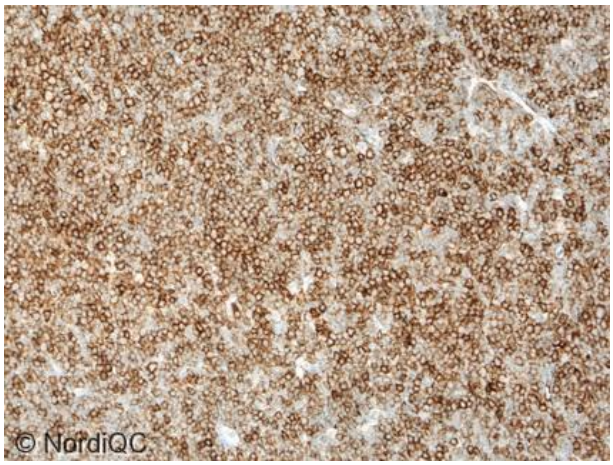


Fig. 3a (x200)
Optimal staining reaction for CD23 of the B-CLL, tissue core no. 4, using same protocol as in Figs. 1a and 2a.
The vast majority of the neoplastic cells show a strong membranous staining reaction – compare with Fig. 3b.

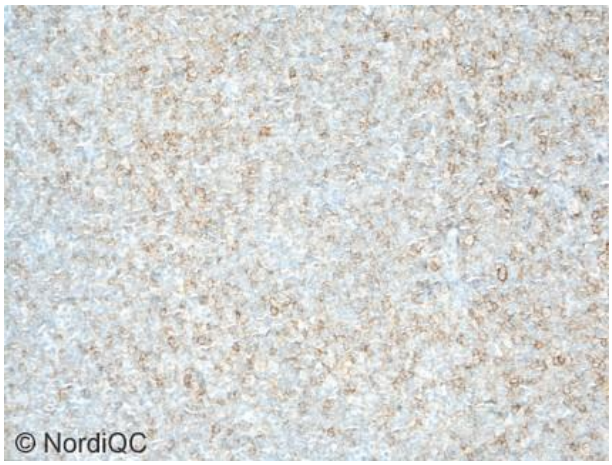


Fig. 3b (x200)
Insufficient staining reaction for CD23 of the B-CLL, tissue core no. 4, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a.
The majority of the neoplastic cells display a reduced staining intensity and a significant proportion of neoplastic cells are false negative.

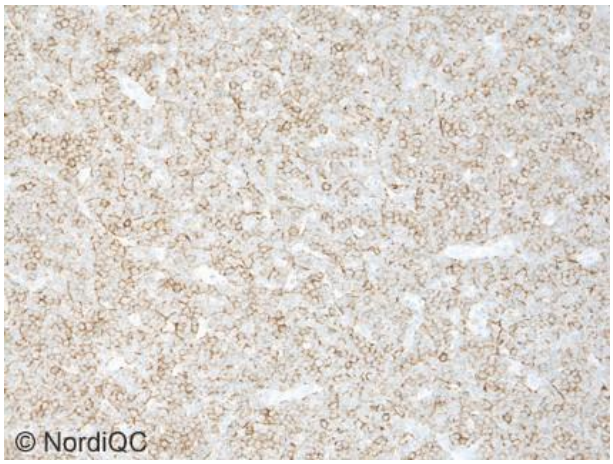


Fig. 4a (x200)
Good staining reaction for CD23 of the B-CLL, tissue core no. 5, using the rmAb clone SP23 in a RTU format (790-4408, Benchmark, Ventana), HIER in CC1 and with a 2-step multimer detection system (UltraView).
Although the majority of the neoplastic cells show a weak to moderate distinct membranous staining reaction, the system can be optimized by using a more sensitive detection system as OptiView– see Fig. 4b.

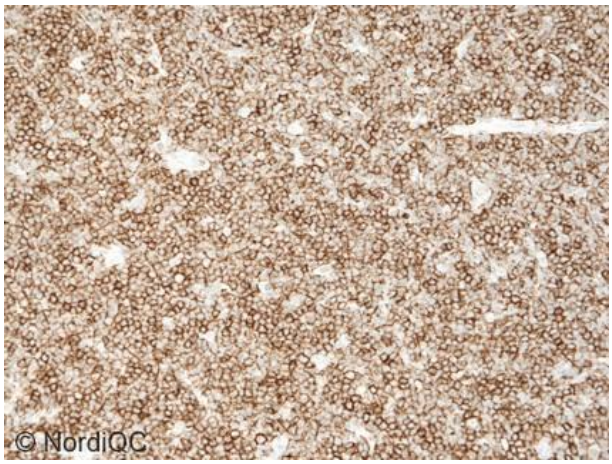


Fig. 4b (x200)
Optimal staining reaction for CD23 of the B-CLL, tissue core no. 5, using the same protocol and RTU format as in Fig. 4a but with a 3-step multimer detection system (OptiView) – same field as in Fig. 4a.
Virtually all neoplastic cells show a strong membranous staining reaction. For this RTU system, the use of 3-step systems, OptiView or UltraView with amplification, significantly increased the proportion of optimal results compared to a 2-step system (UltraView).

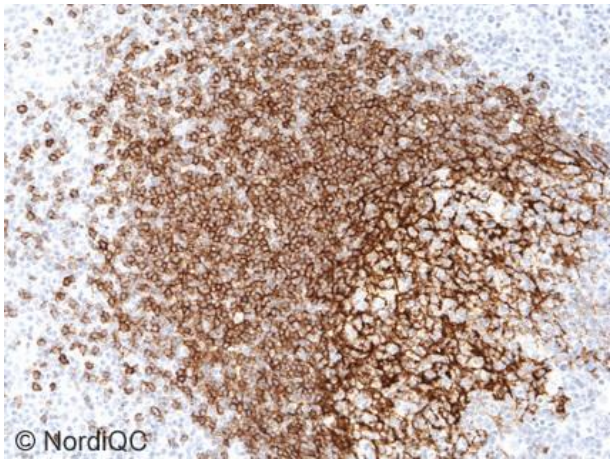


Fig. 5a (x200)
Optimal staining reaction for CD23 of the tonsil using the mAb clone DAK-CD23 (Dako) as concentrate, HIER in an modified acidic buffer (TRS pH 6.1) and a 3-step polymer based detection system (FLEX+, Dako) – same protocol used in Fig. 6a. The B-cells in the mantle zone show a moderate to strong intensity and a distinct membranous staining reaction. - compare with Fig. 5b.

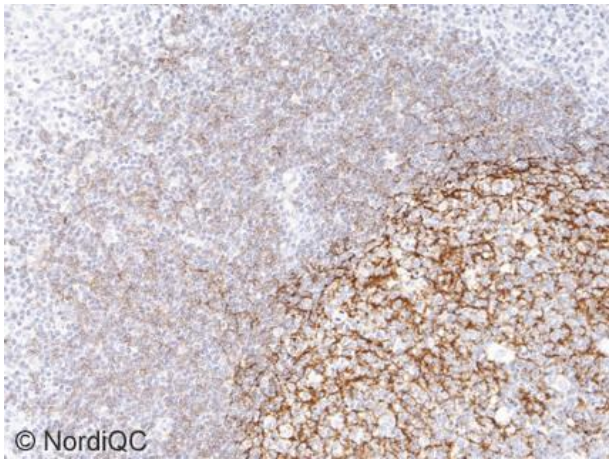


Fig. 5b (x200)
Insufficient staining reaction for CD23 of the tonsil using the mAb clone DAK-CD23 (Dako) as concentrate (too diluted), HIER in alkaline buffer and the use of less sensitive 2-step polymer detection system (REAL EnVision, Dako). The follicular dendritic cells show a moderate staining intensity, but the B-cells in the mantle zone only display a faint or false negative staining reaction. - compare with Fig. 5a.

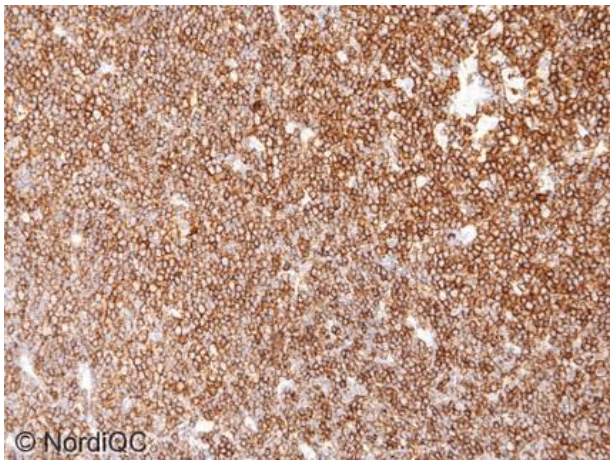


Fig. 6a (x200)
Optimal staining reaction for CD23 of the B-CLL, tissue core no. 4, using the same protocol as in Fig. 5a. Virtually all the neoplastic cells show a strong and distinct membranous staining reaction – compare with the insufficient result in Fig. 6b.

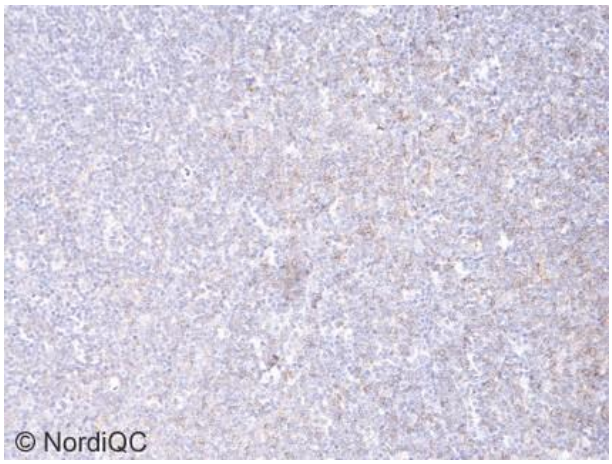


Fig. 6b (x200)
Insufficient staining reaction for CD23 of the B-CLL, tissue core no. 4, using the same protocol as in Fig. 5b. The majority of the neoplastic cells are false negative or only display a faint staining intensity – compare with the optimal result in Fig. 6a.

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