NordiQC

Assessment Run 71 2024

CD20

Purpose
Evaluation of the technical performance, and in particular the level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD20, used for identification of B-cell lymphoproliferative disorders. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD20 (see below).

Material
The slide to be stained for CD20 comprised:
1. Appendix, 2. Tonsil, 3. Malignant melanoma, 4. Diffuse Large B-Cell Lymphoma (DLBCL), 5-6. B-Chronic Lymphatic leukemia (B-CLL)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD20 staining as optimal included:

- A moderate to strong, distinct membranous staining reaction of all mantle zone, germinal centre and interfollicular B-cells in the tonsil and in the appendix.
- A strong, distinct membranous staining reaction of all the neoplastic B-cells in the DLBCL and in the B-CLL (tissue core no. 6).
- A weak to moderate, distinct membranous staining reaction of virtually all the neoplastic B-cells in the B-CLL (tissue core no. 5).
- No staining reaction of other tissue/cell structures including T-cells (all specimens), epithelial cells of the appendix and the neoplastic cells of the malignant melanoma (few dispersed normal B-cells intermingling between the malignant cells should be demonstrated).

KEY POINTS FOR CD20 IMMUNOASSAYS
- The mAb clone **L26** was used by 97% of all participants.
- RTUs developed for the Autostainer, BOND and Benchmark platforms gave superior results applying vendor recommended protocol settings
- The performance of the mAb clone L26, both as concentrate and RTU, was less successful on the Omnis platform
- Tonsil and appendix are not reliable tissue controls to monitor the accuracy and precision of CD20 IHC assays.

Participation

Number of laboratories registered for CD20, run 71	466
Number of laboratories returning slides	428 (92%)

All slides returned after the assessment were assessed and participants received advices if the result was insufficient - data from all these outcomes were not included in this report.

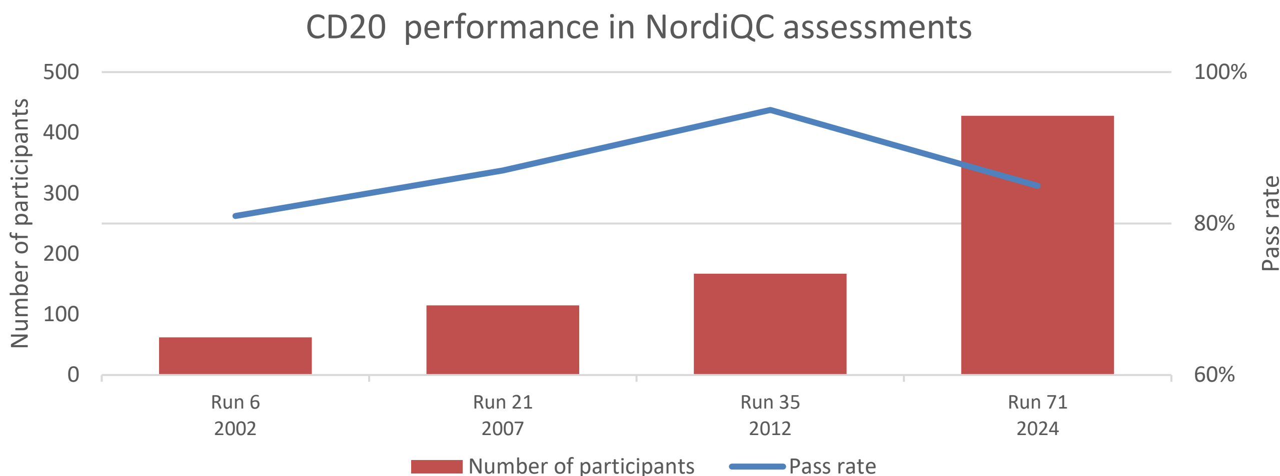
Results
428 laboratories participated in this assessment and 362 (85%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Table 1b and 1c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of the mAb clone L26 on the Omnis platform (Dako/Agilent)
- Too diluted primary antibody
- Unexplained technical issues

Accredited by DANAK under registration number 616 to proficiency testing

Performance history
This was the fourth NordiQC assessment of CD20. The pass rate decreased significantly compared to the previous run 35, 2012 (see Graph 1).

Graph 1. **Proportion of sufficient results for CD20 in the four NordiQC runs performed**



Controls
Normal tonsil and appendix are recommended as positive and negative tissue controls. Virtually all mantle zone and germinal centre B-cells of secondary follicles must show a moderate to strong membranous staining reaction, whereas non-lineage B-cells as T-cells, stromal and epithelial cells must be negative (CD20 is down-regulated in plasma cells and normally negative but might rarely display faint membranous staining reactions).
As no normal tissue structures or B-cells display low level of detection, laboratories should consider to include lymphomas/leukemias with low antigen densities for CD20, e.g., B-CLL`s, both in the validation process to ensure that the protocol provides the correct level of analytical sensitivity, but also in the daily routine to monitor the reproducibility of the CD20 IHC assay. As seen in this run normal tissue with high level CD20 expression as tonsil cannot be used to evaluate IHC assay accuracy and precision.

Conclusion
The widely used mAb clone **L26**, but also the newly introduced clones **MX003**, **QR094, SP32, ZR243, DA144, SDT-R133 and 356D7B4,** could all be used to produce optimal staining results for CD20. Irrespective of the clone applied, efficient HIER, use of a sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. Among Ready-to-Use (RTU) systems from the major vendors, and applying vendor recommended protocol settings, the RTU system **PA0200/PA0359** (Leica Biosystems), **760-2531** (Ventana/Roche) and **IR604** (Dako/Agilent), all based on mAb clone **L26**, provided superior performance with a pass rate of 100% (90/90) - 99% (89/90) being optimal. The Dako/Agilent RTU product **GA604** also based on the mAb clone **L26**, provided the lowest proportion of sufficient and optimal results.

Table 1a. **Overall results for CD20, run 71**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	113	77	19	16	1	85%	68%
Ready-To-Use antibodies	315	236	30	47	2	84%	75%
Total	428	313	49	63	3		
Proportion		73%	12%	15%	1%	85%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CD20, run 71**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L26**	89 8 5 2 1 1 1	Dako/Agilent Leica Biosystems Cell Marque ZytoMed Systems Biocare Medical Diagnostic Biosystems Epredia	73	17	16	1	84%	68%
mAb clone **IHC532**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **EP459Y**	1	Abcam	0	1	0	0	-	-
rmAb clone **QR094**	1	Quartett	1	0	0	0	-	-
rmAb clone **SP32**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **ZR243**	1	Zeta Corporation	1	0	0	0	-	-
pAb clone **PA5-16701**	1	Invitrogen	0	1	0	0	-	-
Total	113		77	19	16	1	-	
Proportion			68%	17%	14%	1%	85%	

1) Proportion of sufficient stains (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results.

Table 1c. **Ready-To-Use antibodies and assessment marks for CD20, run 71**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **L26** **PA0200/PA0359**[3]	19	Leica Biosystems	19	0	0	0	100%	100%
mAb clone L**26** **PA0200/PA0359**[4]	11	Leica Biosystems	11	0	0	0	100%	100%
mAb clone **L26** **IR604**[3]	13	Dako/Agilent	12	1	0	0	100%	92%
mAb clone **L26** **IR604**[4]	13	Dako/Agilent	7	3	3	0	77%	54%
mAb clone **L26** **GA604**[3]	44	Dako/Agilent	8	12	23	1	45%	18%
mAb clone **L26** **GA604**[4]	32	Dako/Agilent	4	8	20	0	38%	13%
mAb clone **L26** **760-2531**[3]	58	Ventana/Roche	58	0	0	0	100%	100%
mAb clone **L26** **760-2531**[4]	110	Ventana/Roche	103	6	1	0	99%	94%
mAb clone **L26** **120M-87/88/80**	3	Cell Marque	3	0	0	0	-	-
mAb clone L**26** **MAD-002037QD**	3	Master Diagnostica	3	0	0	0	-	-
mAb clone **L26** **8259-C010**	3	Sakura FineTek	3	0	0	0	-	-
mAb clone **L26** **PM004AA/H**	1	Biocare Medical	1	0	0	0	-	-
mAb clone **MX003** **MAB-0669**	1	Fuzhou Maixin	1	0	0	0	-	-
mAb clone **CD20/C23** **AM537**	1	BioGenex	0	0	0	1	-	-
rmAb clone **SDT-R133** **I12212E-05**	1	Biolynx Biotechnology	1	0	0	0	-	-
rmAb clone **DA144** **DMRD0179**	1	Dartmon Biotechnology	1	0	0	0	-	-
rmAb clone **356D7B4** **PA206**	1	Abcarta/Abcepta	1	0	0	0	-	-
Total	315		236	30	47	2		
Proportion			75%	10%	15%	1%	85%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of CD20, Run 71

Based on five observations/protocols per antibody clone or RTU system applied.

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **L26**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (3/14)*, Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (14/15), Cell Conditioning 1 (CC1; Ventana/Roche) (40/54),Tris-EGTA buffer pH 9 (1/1), TRS pH 6 (3-in-1) (Dako/Agilent) (1/2), Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (12/15), Cell Conditioning 2 (CC2; Ventana/Roche) (1/1) and Citrate buffer pH 6 (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 71 of 81 (88%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for CD20 for the most commonly used antibody concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **L26**	3/6** (50%)	-	0/8 (0%)	1/2	34/38 (89%)	1/1	10/10 (100%)	11/13 (85%)

* 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)
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra.
3) Bond III.

Ready-To-Use antibodies and corresponding systems
mAb clone **L26**, product no. **PA0200/PA0359**, Leica Biosystems, Bond-III/PRIME:
Protocols with optimal results were typically based on HIER using BERS1 or BERS2 (efficient heating time 20 min. at 100°C), 15 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 21 of 21 (100%) laboratories produced an optimal staining result.

mAb clone **L26**, product no. **IR604**, Dako/Agilent, Autostainer:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000/K8002) as detection systems. Using these protocol settings, 14 of 14 (100%) laboratories produced a sufficient staining result – 93% (13/14) being optimal.

mAb clone **L26**, product no. **GA604**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20-30 min. at 97°C), 12.5-20 min. incubation of the primary Ab and EnVision FLEX+ (GV800/823+GV821) as detection systems. Using these protocol settings, 25 of 55 (45%) laboratories produced a sufficient staining result – 20% (11/55) being optimal.

mAb clone **L26**, product no. **760-2531**, Ventana/Roche, BenchMark Ultra/Ultra PLUS/XT/GX:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 24-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab and OptiView (760-700) or UltraView (760-500) as detection system. Using these protocol settings, 123 of 123 (100%) laboratories produced a sufficient staining result – 97% (119/123) being optimal.

Table 3 summarizes 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 according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for CD20 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS48 mAb L26 **IR604**	100% (13/13)	92% (12/13)	88% (7/8)	63% (5/8)
Dako Omnis mAb L26 **GA604**	45% (20/44)	18% (8/44)	39% (12/31)	13% (4/31)
Leica BOND MAX/III/PRIME mAb L26 **PA0200/PA0359**	100% (19/19)	100% (19/19)	100% (11/11)	100% (11/11)
VMS Ultra/PLUS/XT/GX mAb L26 **760-2531**	100% (58/58)	100% (58/58)	100% (109/109)	95% (103/109)

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

Comments
In this fourth assessment of CD20, the prevalent features of an insufficient result was a too weak or false negative staining reaction, observed in 92% (61/66) of the insufficient results. In the remaining five insufficient protocols, unexplained technical issues were often seen in combination with an uneven and/or excessive counter/background staining - compromising interpretation of the specific signal for CD20. Virtually all laboratories were able to demonstrate CD20 in high-level antigen expressing cells, such as normal B-cells in the tonsil and the neoplastic B-cells of the DLBCL. However, demonstration of CD20 in low-level antigen expressing cells as the neoplastic cells of the B-CLL (tissue core no. 5) was more challenging, particularly for laboratories using the Dako Omnis platform (see below).

Used within laboratory developed assays (LD) or Ready-to-Use formats (RTU), the mAb clone **L26** was the most widely used antibody for demonstration of CD20 and applied by 97% (417/428) of all laboratories. Using this antibody within LD-assays (concentrated formats), the pass rate was 84% (90/107) of which 68% (73/107) of the results were assessed as optimal (see Table 1b). Performance characteristic on the respective automatic platforms from the major vendors are outlined in Table 2, and as shown, the protocols applied on fully automated platforms BOND (Leica Biosystems) and Benchmark (Ventana/Roche) provided a high proportion of optimal results using the mAb clone **L26** in the "recommended/optimal" dilution range 1:50-500. Using all protocol settings on these two platforms, the overall pass rate was 93% (79/85) – 79% (67/85) being optimal. Several protocol settings could be used for an optimal result including parameters that normally are considered to provide low analytical sensitivity such as HIER in Low pH buffers (e.g., BERS1, Leica Biosystems) or use of a 2-step detections system (e.g, UltraView, Ventana/Roche) as long as the titer of the primary Ab was calibrated correctly. Six protocols, applied on these two platforms, were assessed as insufficient and the main cause for less successful performance was use of a too diluted working concentration of the primary antibody – main dilution factor of 1:1.183 (range of 1:600-2.000).
In contrast, and for protocols applied on the fully automated instrument Omnis (Dako/Agilent), the pass rate was only 27% (3/11) – 9% (1/11) being optimal. No parameters could be identified unraveling the discrepancy between a sufficient and an insufficient result as protocol settings based on high analytical sensitivity e.g., HIER in High pH, dilution factor of the primary in the "recommended/optimal range" and the use of the sensitive detection system Envision FLEX+, also produced inferior results. The one protocol assessed as optimal was based on HIER in Low pH (30 min. at 97°C), 20 min. incubation time in primary Ab (diluted 1:500) and Envision FLEX+ as the detection system. For all LD-assays applied on the Omnis, the concentrate (mAb clone **L26**) was acquired from Dako/Agilent (M0755), and in relation to lot variations, at present no specific lot number could be identified explaining for the poor performance on the Omnis platform. The interpretation of impact on lot variations was challenged by the low number of data points pr. lot and at least 6 different lot numbers were used in the 11 protocols performed on this platform.
For participants using LD-assays based on the mAb clone **L26** on the semi-automated instrument Autostainer, and compared to protocols applied on the Omnis platform, the performance of assays used on this platform seems more robust (see also performance for the corresponding RTU system IR604, Dako/Agilent), providing a pass rate of 83% (5/6) – 50% (3/6) being optimal. All sufficient results were based on HIER in TRS High pH and optimal results were obtained using a diversity of protocol settings e.g., the primary antibody was diluted 1:200 (2/3) or 1:500 (1/3), applying Envision FLEX or Envision FLEX+, respectively. One protocol was assessed as insufficient (weak staining) using similar protocol settings as the assays giving an optimal mark, and thus, categorized as "unexplained technical issues". The reason for the discrepancy of results achieved on the Autostainer, but also for the BOND and the Benchmark platforms, versus the relatively poor performance on the Omnis is difficult to elucidate upon and rather speculative but could be related to different wash procedures on the instruments and/or the use of the different antigen retrieval buffer or procedures (chemical composition, time and temperature). In order to elucidate on the diverging results on the Dako Omnis platform, a post-assessment test was performed in a NordiQC reference laboratory on the same tissue material that has been circulated to the participants, using the mAb clone **L26** concentrate (M0755) on the Dako Omnis platform. Several tests were performed using EnVision FLEX+ as the detection system, applying different HIER Buffers (TRS Low pH versus High pH), different HIER times at 97°C ranging for 10-30 min., incubation time in primary antibody for 30 min. with an antibody concentration in the range of 1:50-200 diluted in Dako diluent pH 7.3. In this test set-up the best performing protocol was based on a short HIER time in TRS High pH for 10 min. at 97°C. HIER in TRS Low pH provided a weaker intensity and reduced proportion of CD20 stained neoplastic B-cells, especially in the B-CLL tissue core no. 5. The dilution range applied in this test seems of less importance as the staining intensity was equally strong at 1:50 compared to 1:200. If the HIER time in the TRS High pH buffer was prolonged a reduced intensity and proportion of neoplastic cells in the B-CLL's were observed. However, the mAb clone **L26** performed as expected and fully successfully on the Dako Autostainer 48, typically using the same HIER buffer TRS High pH but for 20 min. at 97°C, pointing towards an unidentified and isolated problem on the Omnis platform.

74% (315/428) of the laboratories used an RTU format for demonstration of CD20. This is a significant increase compared to the former run 35, 2012 in which 35% (59/167) of the participants applied a RTU format.
In this assessment, and using vendor recommended protocol settings (VRPS), the RTU systems **PA0200/0359** (Leica Biosystem, Bond platforms), **760-2531** (Ventana/Roche BenchMark platforms) and **IR604** (Dako/Agilent, Autostainer Link 48) all based on the mAb clone **L26**, provided superior results (see Table 3). Grouped together, the pass rate was 100% (90/90) of which 99% (89/90) of the results were assessed as optimal. Using laboratory developed protocol settings (LMPS), and in particular for the RTU systems developed for the BOND and BenchMark platforms, the proportion of sufficient and optimal results were also high e.g., for the Leica Biosystems RTU system PA0200/0359, all results (30/30) were assessed as optimal using either VRPS or LMPS. For all RTU systems, both low and highly sensitive protocol parameters could be used for an optimal demonstration. The RTU systems were shown to be very robust giving reproduceable high quality staining results especially when used as "true plug-and- play systems" for routine purpose due to accurate data sheets concerning recommended protocol settings.
In comparison to all other RTU systems from the major vendors, and despite using VRPS based on high analytical sensitivity, the RTU system **GA604** (Dako/Agilent), also based on the mAb clone **L26** and developed for the Omnis platform, provided inferior results with a pass rate of only 45% (20/44) - 18% of the results (8/44) being optimal. LMPS did not improve performance (see Table 3) and overall same pass rate and proportion of optimal results were obtained. In total twelve protocols gave an optimal result and the protocol parameters providing optimal results are outlined above (see page 4). On par to the data seen for the concentrated format of clone L26, it was difficult to identify any root cause for the insufficient results based on the corresponding RTU format **GA604** for Dako Omnis. Identical protocols based on HIER in High pH (30 min. at 97°C), incubation time in primary antibody for 12.5-20 min. and EnVision FLEX+ as detection system could both provide an optimal result and an insufficient false negative result. Again, the divergent performance could not be related to the use of the registered lot numbers. As such using exactly the protocol settings for optimal performance as described above, the lot number 41582271 was used by 67% (8/12) of the laboratories obtaining an optimal mark, but 69% (25/36) of the protocols were assessed as insufficient applying the same lot number and typically using the same protocol settings giving optimal results.
These data and results are contradictory, and all together with the results obtained in LD-assay, the use of the mAb clone **L26** for demonstration of CD20 seems problematic on the Omnis platform. These outstanding and unresolved questions, needs to be addressed and NordiQC is in contact with the vendor trying to unravel this dilemma/problem. At present, due to this issue, it is difficult for the NordiQC assessors to specify tailored recommendations to participants obtaining an insufficient assessment score in this run when the protocols are performed on the Omnis platform.

This was the fourth assessment of CD20 in NordiQC (see Graph 1). The pass rate decreased to 85% in this assessment compared to 95% in the previous run 35 (2012). In this assessment, the most common cause for an insufficient staining result was related to the use of the mAb clone **L26** on the Omnis platform, accounting for 80% (53/66) of all insufficient results - typically giving false negative or too weak staining reaction in the B-CLL (tissue core no. 5). Using the same antibody on other platforms e.g., BOND (Leica Biosystems), Benchmark (Ventana/Roche) or Autostainer (Dako/Agilent), both as concentrate and RTU, the overall pass rate was 96% (316/328) of which 89% (292/328) were optimal. For users of the Omnis platform, participants with insufficient results should consider substituting this demanding antibody with an alternative for the demonstration of CD20. Importantly, all protocol settings must be carefully calibrated according to the expected reaction patterns in tissues with the diagnostic relevant range of expression. In this context is should be emphasized that no normal tissue with low level CD20 expression has been identified and a need to include e.g., B-CLLs to verify the performance is warranted (see below).

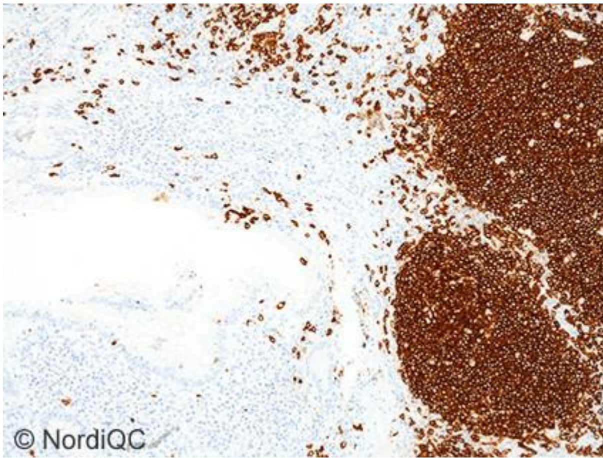


Fig. 1a (x100)
Optimal CD20 staining reaction of the appendix using the RTU system (Dako, GA604) based on mAb clone L26 on the Omnis, following recommendations provided by the vendor: HIER (30 min. at 97°C) in TRS pH High, incubation time in primary Ab (lot 41582271) for 12.5 min and Envision Flex+ as detection system – Same protocol used in Figs. 2a – 6a.
Virtually all mantle zone, germinal centre and dispersed B-cells show a strong, distinct membranous staining reaction. Epithelial cells are negative as expected.

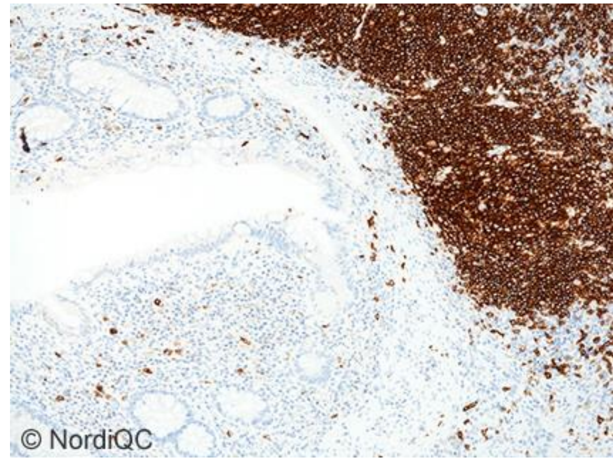


Fig. 1b (x100)
CD20 staining reaction of the appendix using exactly the same RTU system (same lot) and protocol settings as in Fig. 1a, but the protocol gave unexplained insufficient staining reactions (see description of the problem above) – same field as in Fig. 1a. In general, the protocol stained normal B-cells with the expected reaction pattern/level as shown in the Figs. 1a - 4b. However, demonstration of the neoplastic B-cells in the two B-CLL´s were more challenging and especially the B-CLL in tissue core no. 5 (see Figs. 5a – 5b). The conflicting results were seen in many of the protocols based on this RTU system, but also within LD-assays, and no parameter could be identified unraveling for variations in the staining quality. Also, this deviating staining result impacted the overall pass rate for CD20 in this assessment and 80% of the insufficient results, were related to the use of mAb clone L26 on the Omnis platform (both concentrates and the RTU system GA604) – same protocol used in Figs. 2b – 6b.

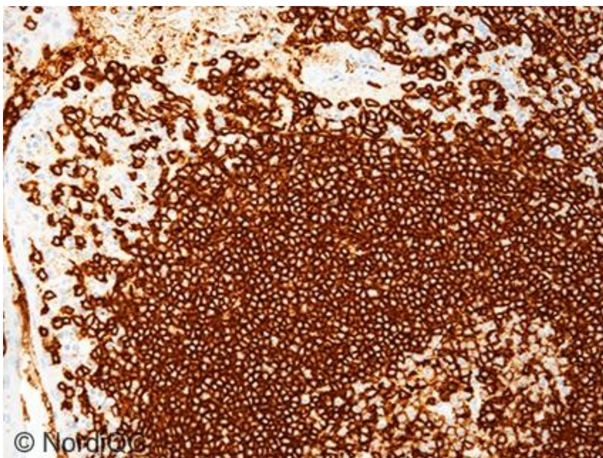


Fig. 2a (x200)
Optimal CD20 staining reaction of the tonsil using same protocol as in Fig. 1a. All B-cells cells display a strong and distinct membranous staining reaction.

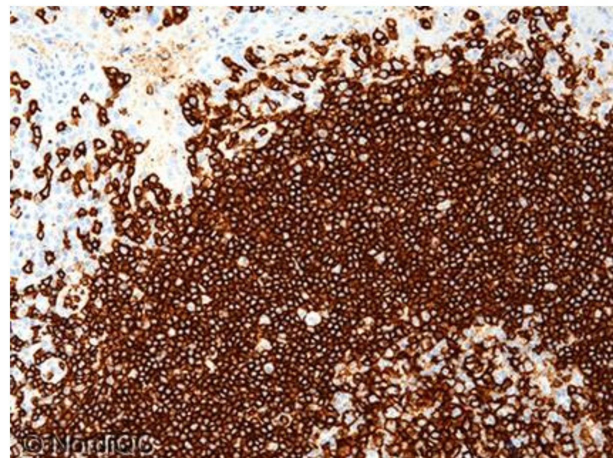


Fig. 2b (x200)
CD20 staining reaction of the tonsil using the same protocol as in Fig. 1b. Note, staining intensity of the B-cells are as strong as the reaction seen in Fig. 2a. This staining pattern might cause problems for many laboratories calibrating the assays for routine purpose as no normal tissue control display low level of expression for CD20. Thus, laboratories are encouraged in the validation process to include lymphomas/leukemias with low antigen densities for CD20, e.g. B-CLL cases, aiming at a protocol providing the correct level of analytical sensitivity for clinical use.

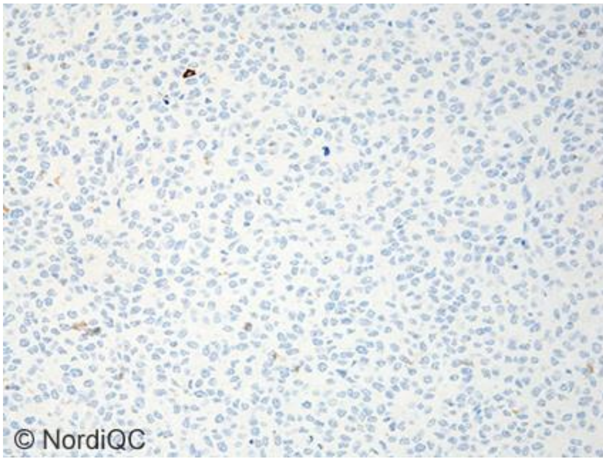


Fig. 3a (x200)
Optimal CD20 staining reaction of the malignant melanoma using same protocol as in Figs. 1a and 2a. All the neoplastic cells are as expected negative, whereas few scattered B-cells are positive.

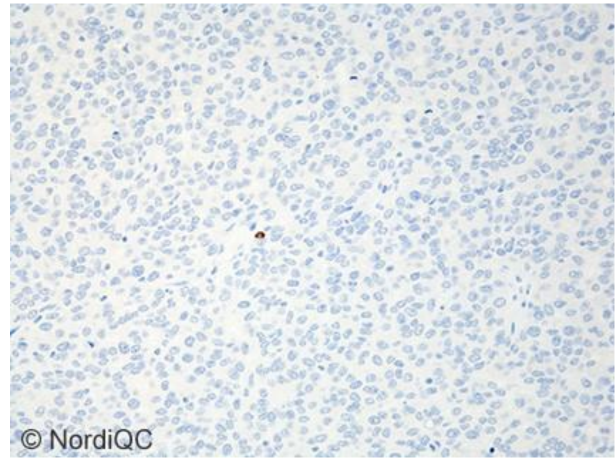


Fig. 3b (x200)
CD20 staining reaction of the malignant melanoma using same protocol as in Figs. 1b and 2b. The protocol gave the same reaction pattern as in Fig 3.a.

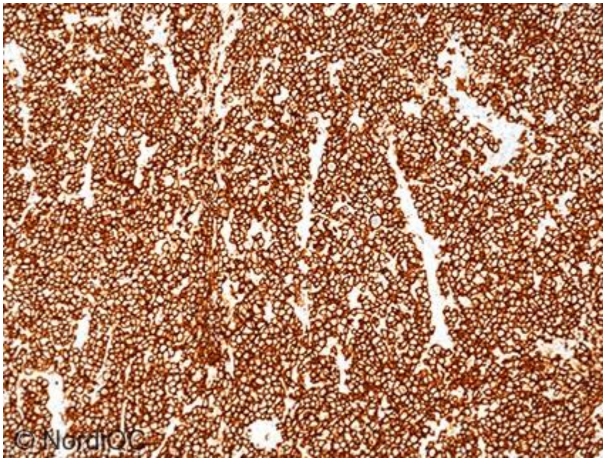


Fig. 4a (x100)
Optimal CD20 staining reaction of the DLBCL using same protocol as in Figs. 1a – 3a. All neoplastic B-cells are strongly positive.

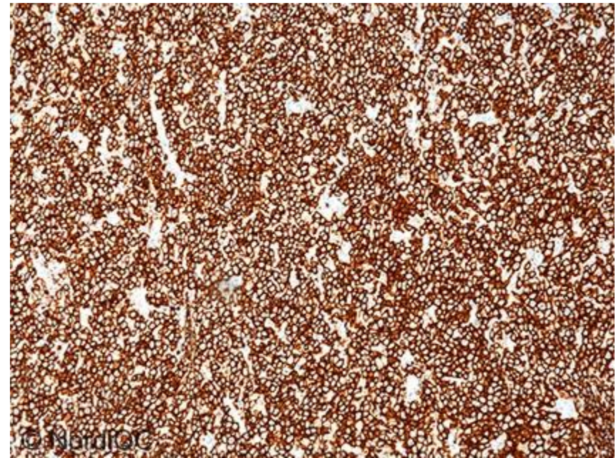


Fig. 4b (x100)
CD20 staining reaction of the DLBCL using same protocol as in Figs. 1b – 3b. In this case, the staining intensity of the neoplastic B-cells are at the same level as seen in Fig. 4a. However, the protocol provided too weak and insufficient staining results in the B-CLL`s below (see Figs. 5a - 6b).

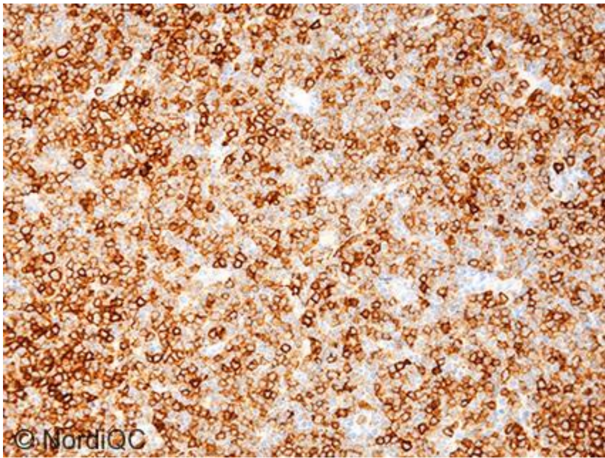


Fig. 5a (x200)
Optimal CD20 staining of the B-CLL (tissue core no. 5) using the same protocol as in Figs. 1a – 4a. Virtually all neoplastic B-cells display a weak to moderate, distinct membranous staining reaction.

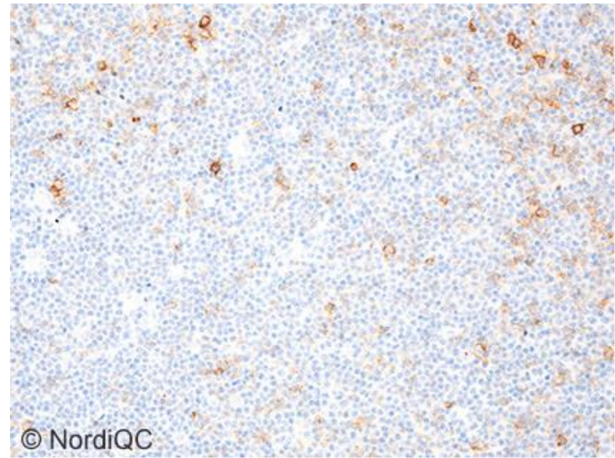


Fig. 5b (x200)
Insufficient CD20 staining reaction of the B-CLL (tissue core no. 5) using same protocol as in Figs. 1b – 4b. The majority of neoplastic B-cells are false negative - compare with optimal results Fig. 5a. As mentioned above, the divergent reaction patterns obtained with this RTU system, and using precisely the same protocol settings, is inexplicable and needs follow-up in cooperation with the vendor.

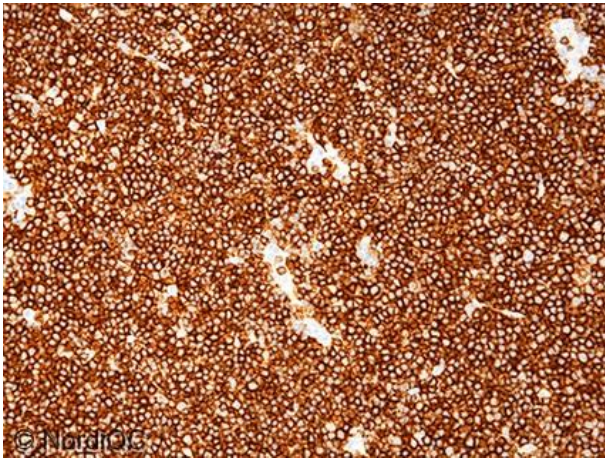


Fig. 6a (x200)
Optimal CD20 staining reaction of the B-CLL (tissue core no. 6) using the same protocol as in Figs. 1a – 5a. All the neoplastic B-cells display a strong staining intensity.

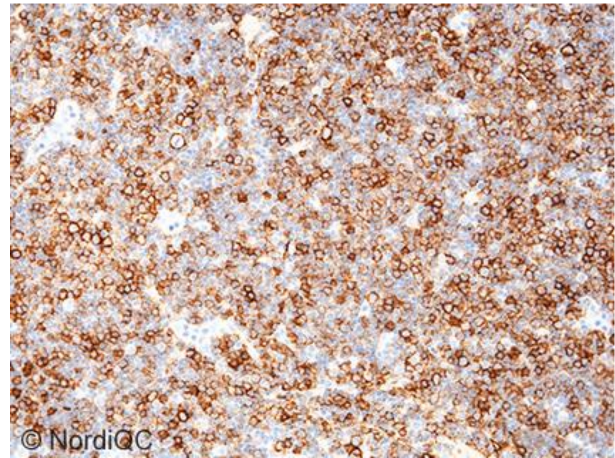


Fig. 6b (x200)
CD20 staining of the B-CLL (tissue core no. 6) using the same protocol as in Figs. 1b – 5b. The proportion of positive neoplastic B-cells and staining intensity is significantly reduced – compare with optimal result in Fig. 6a.

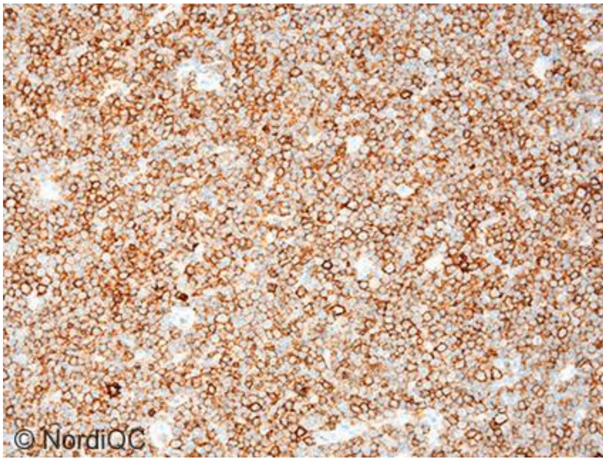


Fig. 7a (x200)
Optimal CD20 staining reaction of the B-CLL (tissue core no. 5) using a LD-assay based on the mAb clone L26 on the BenchMark Ultra platform (Ventana/Roche), efficient HIER in CC1, optimally calibrated primary Ab (1:300) and OptiView as detection system.
All neoplastic B-cells display a weak to moderate membranous staining reaction.

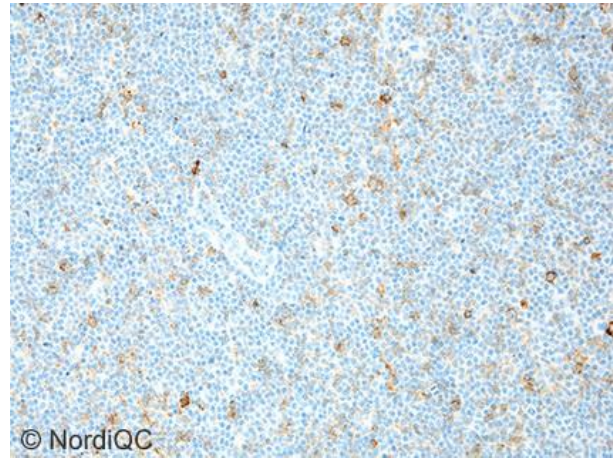


Fig.7b (x200)
CD20 staining reaction of the B-CLL (tissue core no. 5) using an insufficient protocol with too low analytical sensitivity. Protocol settings was nearly identical to the protocol applied in Fig. 7a, but the primary Ab was used in too low concentration (1:2.000). The vast majority of the neoplastic B-cells are false negative.

MB/LE/SN 07.06.24