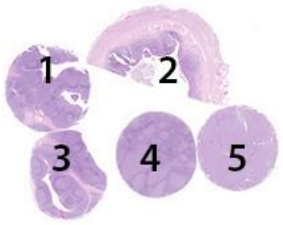
Assessment Run 57 2019

BCL-2 protein (BCL2)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for BCL2 discriminating follicular hyperplasia (reactive lymph nodes) from follicular B-cell lymphomas. Relevant clinical tissue, both normal and neoplastic disorders, was selected displaying a broad spectrum of antigen densities for BCL2 (see below).

Material
The slide to be stained for BCL2 comprised:



1. Tonsil, 2. Appendix, 3. Tonsil, 4. Follicular Lymphoma (FL), grade 2, 5. FL, grade 3*.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing BCL2 staining as optimal included:

- A moderate to strong, predominantly cytoplasmic staining reaction of virtually all T-cells and mantle zone B-cells of follicles in the tonsils and appendix.
- A weak to moderate, distinct cytoplasmic staining reaction of basal squamous epithelial cells in the tonsils and of epithelial cells lining the basal compartment of the crypts in the appendix.
- An at least moderate, distinct cytoplasmic staining reaction of virtually all neoplastic B-cells in the FL grade 2 (tissue core 4).
- A moderate to strong, distinct cytoplasmic staining reaction of the vast majority of neoplastic B-cells in the FL grade 3 (tissue core 5).
- No staining reaction of germinal centre B-cells in the tonsils and luminal epithelial cells of the appendix.

* The FL grade 3 (tissue core 5) displayed rearrangement of the BCL2 gene locus as determined by Fluorescence In Situ Hybridization (FISH), whereas the FL grade 2 (tissue core 4) was normal (negative by FISH).

Participation

Number of laboratories registered for BCl2, run 57	333
Number of laboratories returning slides	319 (96%)

Results
319 laboratories participated in this assessment. 284 (89%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful performance of rmAb clones E17 and EP36
- No pre-treatment or Heat Induced Epitope Retrieval (HIER) in acidic buffer
- Too low concentration of the primary Ab
- Use of less sensitive detection systems
- Unexplained technical issues

Performance history
This was the third NordiQC assessment of BCL2 and, as shown in Table 2, the pass rate increased moderately compared to the latest run 28, 2010.

Table 2. **Proportion of sufficient results for BCL2 in the three NordiQC runs performed**

	Run 13 2005	**Run 28 2010**	**Run 57 2019**
Participants, n=	87	155	319
Sufficient results	93%	82%	89%

Conclusion
The mAb clones **124**, **100/D5**, **BCL2/100/D5, BS94** and the **rmAb** clone **SP66** could all be used to

obtain an optimal staining result. Efficient HIER, preferable in an alkaline buffer, and careful calibration of the antibody titre, in combination with a sensitive and specific IHC system were the main prerequisites for optimal performance. None of the protocols based on the rmAb clones E17 or EP36 were assessed as sufficient. Applying vendor recommended protocol settings (VRPS), the Ready to Use (RTU) systems IS/IR614 (Dako) and PA0117 (Leica) based on the mAb clones 124 and BCL2/100/D5, respectively, both provided a pass rate of 100% and grouped together, 92% were assessed as optimal.
Tonsil is recommendable as positive and negative tissue control for BCL2. All T-cells and B-cells in the mantle zone of the reactive follicles must show a moderate to strong predominantly cytoplasmic staining reaction, whereas the majority of the basal squamous epithelial cells should display a weak to moderate intensity. Germinal centre B-cells should be negative.

Table 1. **Antibodies and assessment marks for BCL2, run 57**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **124**	88 4 2 1 1	Dako/Agilent Cell Marque Diagnostic BioSystems Zeta Corporation EnQuire BioReagents	76	14	4	2	94%	96%
mAb clone **100/D5**	1 1	Biocare Medical Thermo Scientific	0	2	0	0	-	-
mAb clone **BCL2/100/D5**	11	Leica/Novocastra	7	3	1	0	91%	100%
mAb clone **BS94**	1	Nordic Biosite	1	0	0	0	-	-
rmAb clone **SP66**	6	Cell Marque	2	4	0	0	100%	100%
rmAb clone **E17**	2	Cell Marque	0	0	2	0	-	-
rmAb clone **EP36**	1	Cell Marque	0	0	1	0	-	-
Ready-To-Use antibodies								OR[6]
mAb clone **124** **IR/IS614 (VRPS)** [3]	18	Dako/Agilent	17	1	0	0	100%	94%
mAb clone **124** **IR/IS614 (LMPS)** [4]	9	Dako/Agilent	7	2	0	0	100%	-
mAb clone **124** **IR/IS614**[5]	45	Dako/Agilent	32	11	2	0	96%	-
mAb clone **124** **790-4464 (LMPS)** [4]	64	Ventana/Roche	31	24	8	1	86%	-
mAb **124** **226M**	1	Cell Marque	1	0	0	0	-	-
mAb **100/D5** **PM003**	1	Biocare Medical	1	0	0	0	-	-
mAb **BCL2/100/D5** **PA0117 (VRPS)** [3]	7	Leica	6	1	0	0	100%	86%
mAb **BCL2/100/D5** **PA0117 (LMPS)** [4]	5	Leica	5	0	0	0	100%	-
mAb **MX022** **MAB-0711**	1	Maixin	1	0	0	0	-	-
rmAb clone **SP66** **790-4604 (VRPS)** [3]	6	Ventana/Roche	3	2	1	0	83%	50%
rmAb clone **SP66** **790-4604 (LMPS)** [4]	28	Ventana/Roche	12	11	4	1	82%	-
rmAb clone **SP66** **226R-27/28**	7	Cell Marque	2	5	0	0	100%	-
rmAb clone **EP36** **MAD-000675QD**	2	Master Diagnostica	0	0	2	0	-	-
rmAb clone **EP36** **8459-C010**	2	Sakura FineTek	0	0	2	0	-	-
rmAb clone **EP36** **PR004**	1	PathnSitu Biotech.	0	0	1	0	-	-
rmAb clone **EP36** **AN723**	1	BioGenex	0	0	0	1	-	-
rmAb clone **E17**	2	Cell Marque	0	0	2	0	-	-

226R-17/18								
Total	319		204	80	30	5	-	
Proportion			64%	25%	9%	2%	89%	

1) Proportion of sufficient stains (optimal or good). For Laboratory Developed (LD) assays (≥5 asessed protocols)
2) Proportion of sufficient stains with optimal protocol settings only, see below. For LD assays (≥5 asessed protocols).
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 on the vendor recommended platform(s) (≥5 asessed protocols).
5) RTU product developed for a specific semi/fully automated platform by a given manufacturer but inappropriately applied by laboratories on other non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).
6) Proportion of Optimal Results (OR) applying the RTU as recommended by the vendor (≥5 asessed protocols).

Detailed analysis of BCL2, Run 57

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **124**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (27/27)*, Cell Conditioning 1 (CC1, Ventana) (31/45), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (11/11), DBS Montage EDTA Antigen Retrieval Solution (Diagnostic Biosystems)(1/1), Tris-EDTA pH 9 (2/3) or Bond Epitope Retrieval Solution 1 (BERS1, Leica) (4/5) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:100 depending on the total sensitivity of the protocol employed. Using these protocol, settings 73 of 76 (96%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **BCL2/100/D5**: Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (Dako) (2/2), CC1 (Ventana) (2/3), BERS2 (Leica) (1/2) or BERS1 (Leica) (2/4) 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, 6 of 6 (100%) laboratories produced a sufficient staining result (all assessed as optimal).

mAb clone **BS94**: One protocol with an optimal result was based on HIER using Tris-EDTA pH 9 as retrieval buffer. The mAb was diluted 1:300 and HRP-Polymer-anti-mouse (Nordic Biosite) was applied as detection system.

rmAb clone **SP66**: Protocols with optimal results were based on HIER using CC1 (Ventana) (1/4) or BERS1 (Leica) (1/1) as retrieval buffer. The mAb was diluted 1:100 and BOND Refine (Leica) or OptiView (Ventana) were used as detection systems. Using these protocol settings, 2 of 2 (100%) laboratories produced a sufficient staining result (both assessed as optimal).

Table 3. **Proportion of optimal results for BCL2 for the most commonly used antibodies as concentrate on the four 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 **124**	9/9**	0/1	12/12 (100%)	-	28/42 (67%)	0/1	4/4	3/3
mAb clone **BCL2/100/D5**	1/1	-	1/1	-	2/2	-	1/1	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 **124**, product no. **IS/IR614**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-30 min. at 95-97°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Applying vendor recommended protocol settings (VRPS), the proportion of sufficient results (good or optimal) was 100% (18/18) and 94% (17/18) of the laboratories produced an optimal staining result (see Table 1).

mAb clone **124**, product no. **790-4464**, Ventana, BenchMark XT/Ultra:

Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 32-76 min. at 94-100°C), 16-60 min. incubation of the primary Ab and UltraView with amplification (760-500/760-080) or OptiView with or without amplification (760-700/860-099) as detection systems. None of the laboratories applied VRPS (see Table 1).

mAb clone **100/D5**, product no. **PM003**, Biocare Medical, intelliPATH:
One protocol with an optimal result was based on HIER in Reveal Decloaker (efficient heating time 15 min. at 110°C), 45 min. incubation of the primary Ab and MACH4 HRP Polymer (MRH534 + UP534) as detection system.

mAb clone **BCL2/100/D5**, product no. **PA0117**, Leica, BOND III/MAX:
Protocols with optimal results were typically based on HIER in BERS2 (efficient heating time 10-30 min. at 95-100°C), 15-30 min. incubation of the primary Ab and BOND Refine (DS9800) as detection system. Applying VRPS, the proportion of sufficient results (good or optimal) was 100% (7/7) and 86% (6/7) of the laboratories produced an optimal staining result (see Table 1).

rmAb clone **SP66**, product no. **790-4604**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 24-64 min. at 94-100°C), 16-60 min. incubation of the primary Ab and OptiView (760-700) as detection system. Applying VRPS, the proportion of sufficient results (good or optimal) was 83% (5/6) and 50% (3/6) of the laboratories produced an optimal staining result (see Table 1).

Comments
In concordance with the previous NordiQC assessments for BCL2, the prevalent feature of an insufficient staining result was a too weak or false negative staining reaction of cells and tissue structures expected to be demonstrated. This pattern was observed in 94% of the insufficient results (33/35). The remaining insufficient results were characterized by poor signal-to-noise ratio and/or impaired morphology compromising interpretation. Virtually all laboratories were able to stain BCL2 in high-level antigen expressing cells as T-cells in the interfollicular zones and mantle zone B-cells of reactive follicles in the tonsils or neoplastic B-cells of the FL grade 2 (tissue core 4), whereas demonstration of BCL2 in intra-germinal centre T-cells, basal squamous epithelial cells in the tonsils, columnar cells lining the basal compartment of the crypts in the appendix and neoplastic B-cells of the FL grade 3 (tissue core 5) was more challenging, requiring appropriate protocol settings for optimal performance.

FLs are typically characterized by the translocation t(14;18)(q32;q21) resulting in constitutive overexpression of BCL2. For participants using either the rmAb clones E17 or EP36 (same antibody according to *Lin F et al.: Handbook of practical immunohistochemistry: Frequently asked questions; Springer Science+Business Media New York 2015, chapter 7.10, p83),* none (11/11) of the assays based on these clones could provide a sufficient staining result characterized by false negative staining of the neoplastic B-cells in the FL grade 3 (tissue core 5). This atypical staining pattern is difficult to elucidate upon as this FL was tested positive for rearrangement of the BCL2 gene by FISH. In contrary, and as described in the literature (*Hum Pathol. 2013 Sep;44(9):1817-26*), the rmAb E17 (alias EP36) should in fact provide superior/improved sensitivity, detecting overexpression of the BCL2 protein caused by rearrangements of the BCL2 gene locus, otherwise unreactive by immunohistochemistry with the "classical" mAb clone 124. However, biological deviation of the BCL2 protein exist and could explain for lack of reaction with the rmAb clones E17 or EP36 e.g. the epitope sequence identified by the primary Ab may be or is partly truncated due to "cryptic"/aberrant rearrangement of the BCL2 gene locus. The different expression patterns, depending on the clone applied, are illustrated in Fig. 5a-6b. Due to the contradictive results from this assessment and what has been described in the literature to this subject, it is difficult to advise laboratories in regard to the choice of the primary antibody, other than adding additional robust BCL2 clones , e.g. mAb BCL2/100/D5 and/or rmAb SP66, to the portfolio of primary Abs for diagnosis of FL. This strategy could minimize the number of "BCL2 IHC false negative FLs". Overexpression of BCL2 in the neoplastic B-cells of the FL grade 2 (tissue core 4) could be detected with all primary antibodies applied inclusive the rmAb clones E17/EP36, although the tumor cells lack FISH evidence of rearrangement of the BCL2 gene locus. This reaction pattern is not uncommon and Leich E et al.(Blood. 2009 Jul 23;114(4):826-34) demonstrated, that a significant proportion of t(14;18) negative FLs (35%) were positive for BCL2 expression as determined by immunohistochemistry.

Used within a LD assay, the mAb clone 124 was the most widely used antibody for the demonstration of BCL2 providing an overall pass rate of 94% (90/96) (see Table 1). Only six protocols provided an insufficient result typically caused by omission of pre-treatment, HIER in acidic buffer, too diluted primary ab or a too low sensitive detection system. These parameters providing reduced analytical sensitivity were either applied alone or in combination. As shown in Table 3, optimal results could be obtained on all four

main IHC platforms. Applying optimal protocol settings based on HIER in an alkaline buffer, all slides stained on the platforms from Dako (Autostainer/Omnis) and Leica (BOND III/MAX) were assessed as optimal (25/25), whereas the proportion of optimal results were significantly reduced on the Benchmark instruments (Ventana). As described in the previous report (Run 28, 2010), and known from NordiQC reference laboratories, the mAb clone 124 can be challenging on Benchmark platforms requiring highly sensitive protocol settings for optimal performance - typically using efficient HIER in CC1 (e.g. 32-76 min. at 96-100°C), high concentration of the primary ab (dilution range 1:10-1:25) and a sensitive detection system as the Optiview with or without amplification. All protocols applying these settings were assessed as sufficient (11/11) and 91% (10/11) were giving an optimal mark. In support of this observation, and applying highly sensitive protocol settings (efficient HIER in an alkaline buffer and a 3-step multimer/polymer detection system), the Average Dilution Factor (ADF) for optimal performance was 1:39 if mAb clone 124 was used on the BenchMark (Ventana) instrument, whereas the ADF was 1:88 and 1:224 on the fully automated platforms Omnis (Dako) and BOND (Leica), respectively.

The performance of the mAb clone BCL2/100/D5 used within a LD assay was very good and, as shown in Table 1 & 3, provided a high pass rate and proportion of optimal results. The single protocol assessed as insufficient used the primary Ab too diluted (1:200) on the Ventana Benchmark Ultra at a concentration, that was 10-20 times/fold lower compared to protocols providing optimal results (1:10-1:25) on the same instrument.

63% (200/319) of the participants used a RTU system for detection of BCL2. For the RTU system IS/IR614 (Dako, Autostainer) based on the mAb clone 124, the official vendor recommended protocol settings (VRPS) provided high proportions of sufficient and optimal results, 100% (18/18) and 94% (17/18), respectively. Laboratory modified protocol settings (LMPS) to the RTU product also gave high proportions of sufficient and optimal results (see Table 1), typically adjusting HIER time, incubation time in primary Ab or use of a more sensitive detection system (EnvFlex+ versus EnvFlex). A significant number of laboratories (63%, 45/72) used the RTU product IS/IR614 developed for the semi-automated platform Autostainer by Dako off-label. 78% (35/45) of the users applied the RTU product on the Omnis instrument (Dako) of which 97% (34/35) produced a sufficient result and 69% (24/35) were optimal. The proportion of optimal results was highly influenced by the choice of the selected detection system. Protocols based on Envision Flex+ (GV800/823+GV21) was most successful and provided a proportion of 92% (22/24) optimal results, compared to 18% (2/11) for Envision Flex (GV800/823) (all other protocol settings not being addressed).

None of the laboratories (64/64) using the RTU system 790-4464 (BenchMark GX/XT/Ultra, Ventana), also based on the mAb clone 124, followed the official VRPS as given in the package insert (see Table 1). Using LMPS, none of parameters related to HIER (buffer, time temperature) or incubation time of the primary Ab could unravel differences in the performance of the assays. The most important and critical parameter impacting the overall level of analytical sensitivity was the choice of the selected multimer detection system. If OptiView with or without amplification (760-700/760-099) was applied, the proportion of sufficient results was 100% (32/32) of which 78% (25/32) were assessed as optimal. UltraView with amplification (760-500/760-080) provided 85% (11/13) sufficient results of which 38% (5/13) were optimal, whereas the use of UltraView (760-500) without amplification provided an inferior performance as the pass rate declined to 63% (10/16) and none was assessed as optimal.

The RTU system PA0117 (Leica) based on the mAb clone BCL2/100/D5 also provided a high proportion of sufficient and optimal results (see Table 1). For participants strictly following the recommendations given by the vendor, the pass rate was 100% (7/7) of which 86% (6/7) were assessed as optimal (see Table 1). For laboratories modifying protocol settings, typically adjusting the time in HIER and incubation with primary Ab, all (5/5) obtained an optimal mark.

In this assessment, the RTU system from Ventana (790-4604) based on the rmAb SP66 provided an overall pass rate of 82% (28/34) (see Table 1). Only 18% (6/34) of the laboratories followed the official protocol recommendations from the vendor of which 50% (3/6) were assessed as optimal (see Table 1). Of all protocols assessed as optimal, both VRPS and LMPS, 93% (14/15) were based on OptiView (with or without amplification) as detection system. If protocols were based on UltraView (with or without amplification), only one (1/14) provided an optimal mark. In general, and also observed with concentrated format of this clone, the FL grade 3 (tissue core 5) was challenging requiring assays with a high analytical sensitivity. As for the RTU 790-4464 (mAb clone 124), it was difficult to identify other critical parameters impacting the overall performance of the assays other than the choice of the detection system.

This was the third assessment of BCL2 in NordiQC (see Table 2). The pass rate increased to 89% compared to a pass rate of 82% in the previous run 28, 2010. The most important parameters influencing the final outcome in negative direction was: 1) The use of the rmAb clone E17/EP36 providing false negative result of the neoplastic cells in the FL grade 3 (tissue core 5). In total, 31% (11/35) of the insufficient results were based on protocols using one of these antibodies, 2) The use of protocols with too low analytical sensitivity for otherwise successful Abs as e.g. mAb clone 124 and in particular the use of 2-step detection systems which were less successful compared to more sensitive 3-step polymer/multimer detection systems. Importantly, laboratories should use a robust Ab, calibrate the protocols correctly and stain according to the expected antigen level of the recommended control material (see below).

Controls

Tonsil is recommendable as positive and negative tissue control for BCL2. A moderate to strong, predominantly cytoplasmic staining reaction should be displayed in virtually all T-cells and B-cells in the mantle zone of the reactive follicles, whereas the majority of the basal squamous epithelial cells, e.g. lining the tonsillar crypts, must show an at least weak staining intensity. Germinal centre T-cells should be distinctively demonstrated, whereas germinal centre B-cells should be negative.

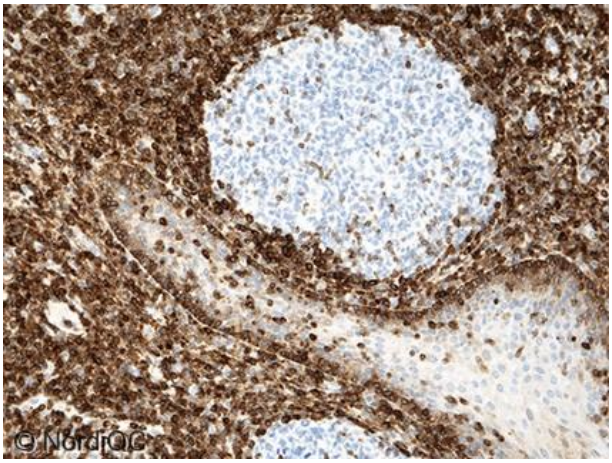


Fig. 1a (x200)
Optimal BCL2 staining of the tonsil using the mAb 124 optimally calibrated, HIER in an alkaline buffer (TRS pH 9, Dako) and Envision Flex+ (Dako) as the detection system - same protocol used in Figs. 2a-4a.Virtually all the peripheral B- and T-cells show a strong staining. In the germinal centres, scattered T-cells show a distinct reaction, whereas the B-cells are negative. The basal squamous epithelial cells display moderate staining intensity.

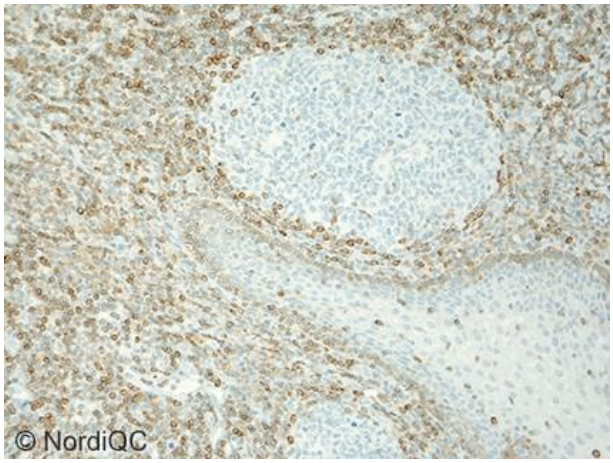


Fig. 1b (x200)
Insufficient BCL2 staining of the tonsil using the mAb 124 in the optimal dilution range but with a protocol providing low analytical sensitivity, no pre-treatment and the low sensitive UltraView (Ventana) as the detection system - same protocol used in Figs. 2b-4b. The peripheral B- and T-cells show too weak staining intensity and intra-germinal T-cells (internal control to the negative germinal centre B-cells) are only faintly demonstrated or false negative. Although the basal squamous epithelial cells are stained, the intensity and overall analytical sensitivity is reduced and critical in relation to neoplastic disorders – compare Fig. 1a-4b.

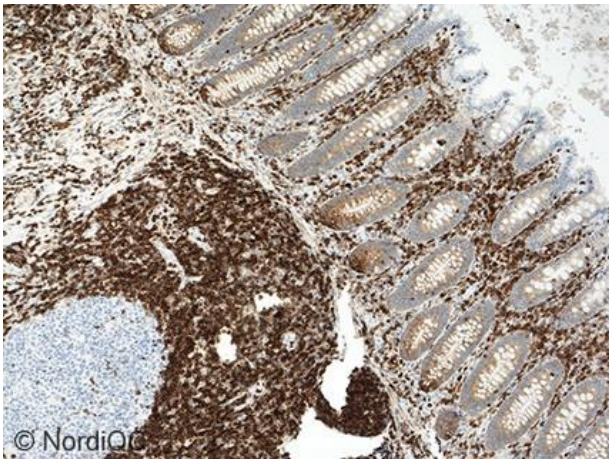


Fig. 2a (x100)
Optimal BCL2 staining of the appendix using same protocol as in Fig. 1a. The B-and T-cells show the expected strong distinct cytoplasmic reaction pattern (germinal centre B-cells are negative), while virtually all columnar cells in the basal compartment of the crypts show an at least weak predominantly cytoplasmic staining reaction.

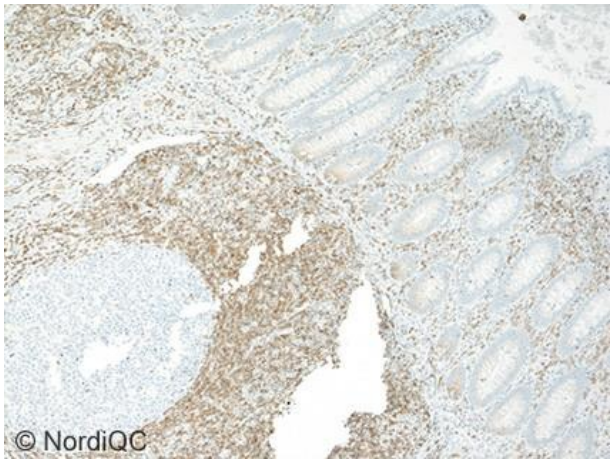


Fig. 2b (x100)
Insufficient BCL2 staining of the appendix using same protocol as in Fig. 1b. The B-and T-cells show a too weak staining intensity and the vast majority of epithelial cells lining the basal compartment of the crypts are false negative or only faintly demonstrated - compare with Fig. 2a.

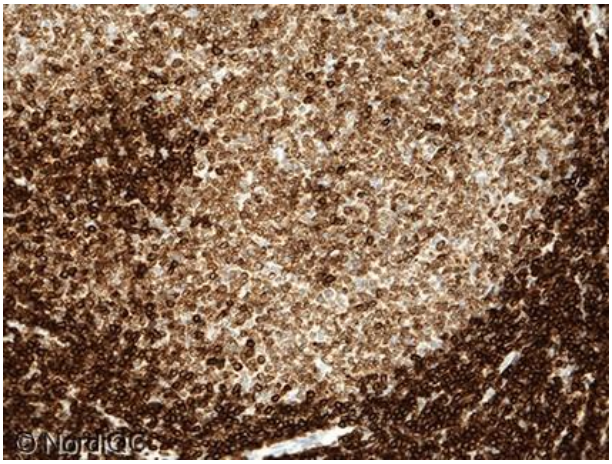


Fig. 3a (x200)
Optimal BCL2 staining of the FL grade 2 (tissue core 4) using same protocol as in Figs. 1a-2a. Virtually all neoplastic B-cells show a moderate to strong predominantly cytoplasmic staining reaction, while all normal lymphocytes display strong staining intensity.

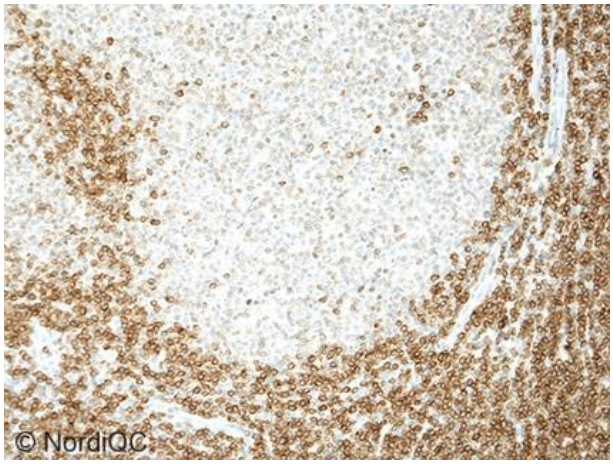


Fig. 3b (x200)
Insufficient and false negative BCL2 staining of the FL grade 2 (tissue core 4) using same protocol as in Figs. 1b-2b. The proportion of stained neoplastic B-cells is significantly reduced, and intensity is too weak. Only the normal lymphocytes are demonstrated - compare with Fig. 3a.

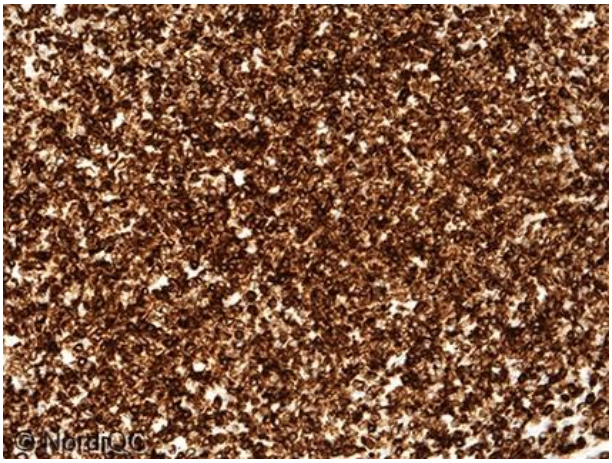


Fig. 4a (x200)
Optimal BCL2 staining of the FL grade 3 (tissue core 5) using same protocol as in Figs. 1a-3a. Virtually all neoplastic cells show a strong, distinct cytoplasmic staining reaction.

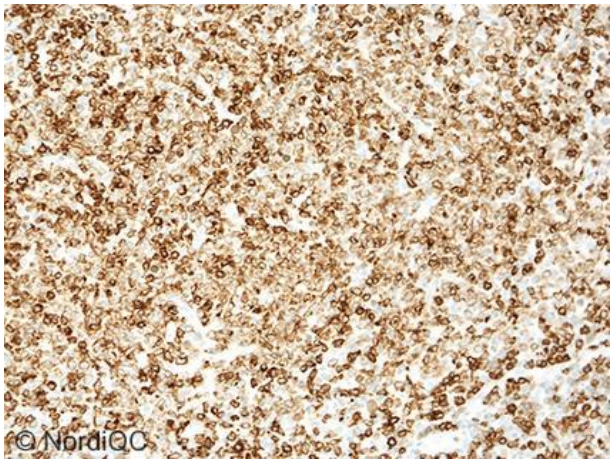


Fig. 4b (x200)
Insufficient BCL2 staining of the FL grade 3 (tissue core 5) using same protocol as in Fig. 1b-3b. Although the neoplastic cells are positive, the protocol provides an overall too low sensitivity due to the false negative staining reaction of the neoplastic cells in the FL grade 2 (tissue core 4). – compare Figs. 3a-4b.

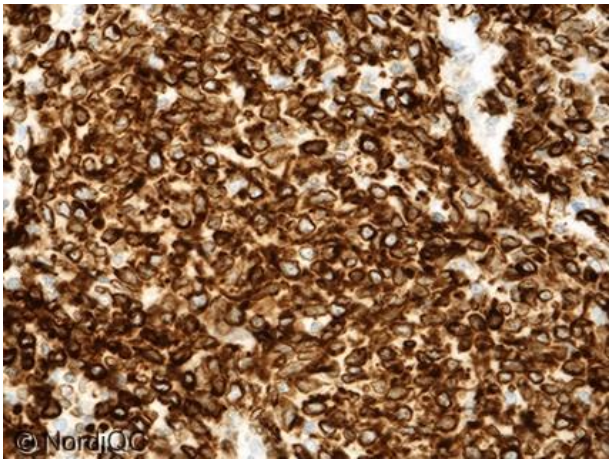


Fig. 5a (x400)
BCL2 expression of the FL grade 3 (tissue core 5) using the mAb 124 stained in a NordiQC reference laboratory. All neoplastic cells display a strong, distinct cytoplasmic staining reaction – compare the expression patterns of BCL2 depending on the selected antibody clones in Fig. 5a-6b.

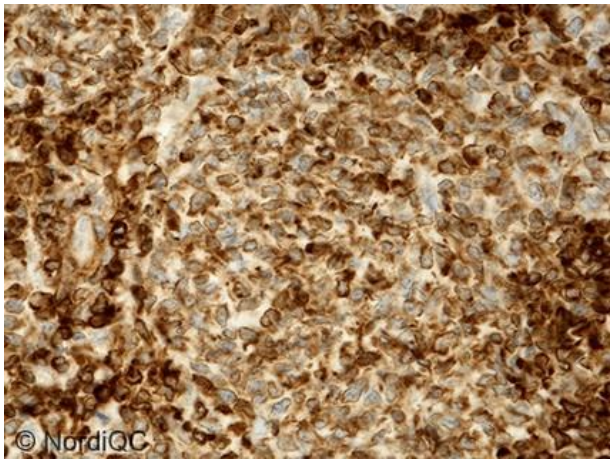


Fig. 5b (x400)
BCL2 expression of the FL grade 3 (tissue core 5) using the rmAb SP66 stained in a NordiQC reference laboratory. The staining intensity of the neoplastic B-cells is weaker, but still in the optimal range, compared to the intensity obtained with the mAb 124 in Fig. 5a. Protocols based on SP66 required settings with a high analytical sensitivity e.g. efficient HIER in alkaline buffer and a sensitive 3-step multimer/polymer detection system as OptiView.

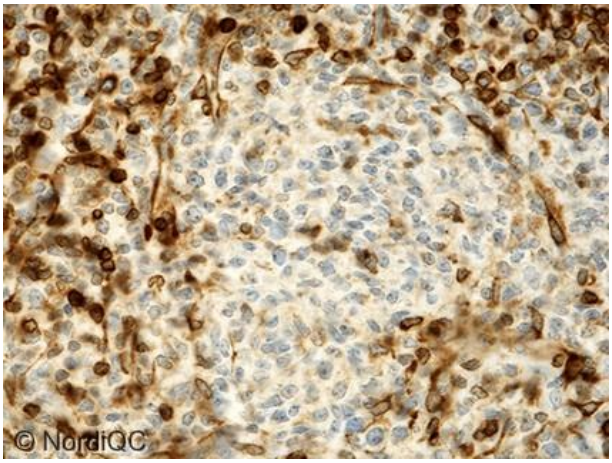


Fig. 6a (x400)
Insufficient BCL2 staining of the FL grade 3 (tissue core 5) using the rmAb E17 (alias EP36) stained in a NordiQC reference laboratory. All neoplastic B-cells are negative – compare with Fig 5a-5b. Only normal lymphocytes and stromal cells display distinct cytoplasmic staining reaction (see discussion in comments).

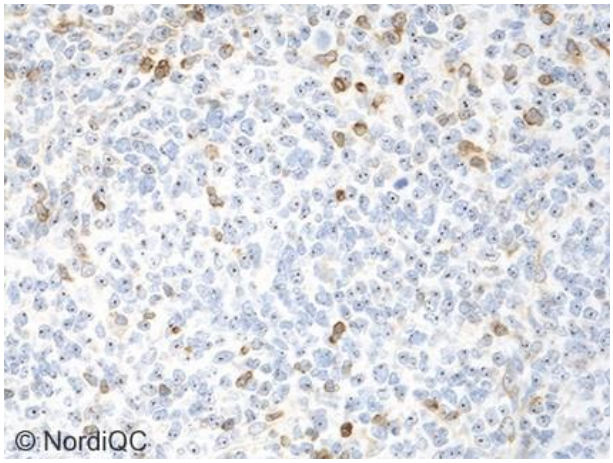


Fig. 6b (x400)
Insufficient BCL staining of the FL grade 3 (tissue core 5) using the rmAb E17 on the BOND platform (Leica). All neoplastic cells are false negative but displayed an aberrant staining reaction of nucleoli in virtually all tumor cells. This pattern was frequently seen when the primary Ab was applied on either BOND or Autostainer (Dako) platforms.

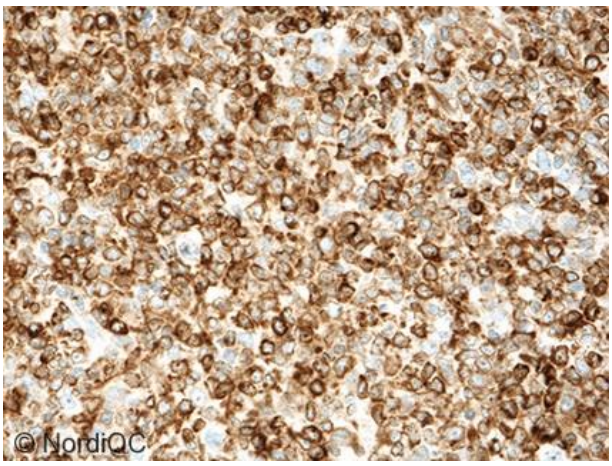


Fig. 7a (x400)
Optimal BCL2 staining of the FL grade 3 (tissue core 5) using the RTU 790-4604 based on the rmAb SP66 on the Benchmark Ultra (Ventana), HIER in CC1 (64min/100°C), incubation in primary Ab (52 min/36°C) and **OptiView** as detection system. The protocol provided the required analytical sensitivity and all neoplastic B-cells display a moderate to strong, distinct cytoplasmic staining reaction.

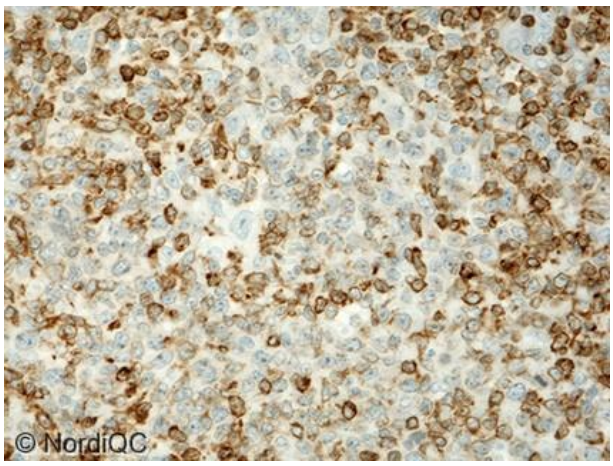


Fig. 7b (x400)
Insufficient BCL2 staining of the FL grade 3 (tissue core 5) using the RTU 790-4604 based on the rmAb SP66 on the Benchmark Ultra (Ventana) applying a protocol with too low analytical sensitivity, HIER in CC1 (60min/100°C), incubation in primary Ab (32 min/36°C) and **UltraView** as detection system. The vast majority of the neoplastic B-cells are all false negative or only faintly demonstrated – compare with optimal protocol in Fig. 7a.

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