Assessment Run 73 2025

BCL-2 protein (BCL2)

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

Material
The slide to be stained for comprised:
1. Tonsil, 2. Appendix, 3. Spleen, 4-5. Follicular B-cell Lymphomas

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a BCL2 staining as optimal included:

- A moderate to strong, predominantly cytoplasmic staining reaction of T-cells in the interfollicular zones, all mantle zone B-cells and intra-germinal centre T-cells of secondary follicles in the tonsil.
- An at least weak, but distinct staining reaction of virtually all littoral cells lining sinusoids in the spleen (red pulp), whereas lymphatic cells of the white pulp displaying a strong staining intensity.
- A strong, distinct staining reaction of virtually all neoplastic B-cells in the two follicular lymphomas.
- No staining reaction of activated intra-germinal centre B-cells in the tonsil, smooth muscle and luminal epithelial cells of the appendix.
- Basal and intermediate epithelial cells of the crypts and enteric neurons/ganglion cells intermingling between smooth muscle cells of lamina muscularis propria of the appendix showing an at least weak to moderate staining reaction.

KEY POINTS FOR BCL2 IMMUNOASSAYS

- The mAb clones **124, BCL-2/100/D5** and the rmAb clone **SP66** were used by 95% of all participants, providing a cumulated high pass rate of 93%.
- The rmAb clones **E17** and **EP36** gave an aberrant staining reaction of nucleoli.
- The most common parameter influencing performance in negative direction was use of low sensitive detection systems.
- RTU systems from the major vendors using recommended protocol settings were successful for demonstration of BCL2, giving 97% sufficient results.
- Among RTU systems, **PA0117** (Leica Biosystems) mAb clone **BCL-2/100/D5** and **790-4604** (Ventana/Roche) rmAb clone **SP66**, provided superior performance with 100% sufficient results.
- Littoral cells lining spleen sinusoids seemed to be more reliable as positive critical control tissue compared to basal squamous epithelial cells in tonsil.

Participation

Number of laboratories registered for BCL2, run 73	458
Number of laboratories returning slides	437 (95%)

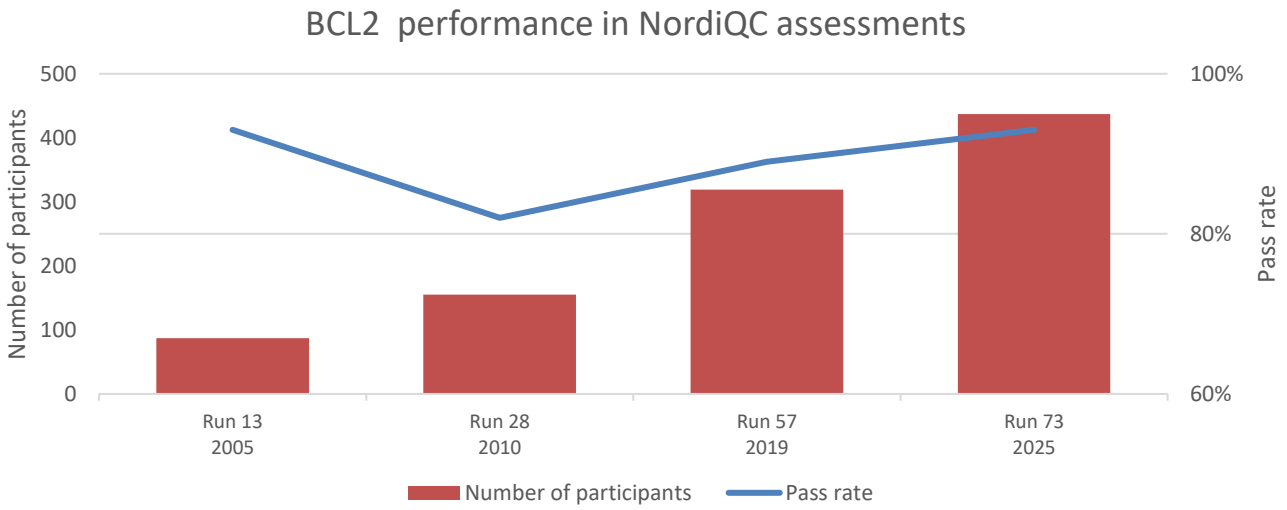
All slides returned after the assessment were assessed and participants received advice if the result was insufficient - data from all these outcomes were not included in this report.

Results
437 laboratories participated in this assessment and 406 (93%) 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:
- Use of inefficient HIER in an acidic buffer or too short HIER time
- Use of less sensitive 2-step detection systems e.g. UltraView (Ventana/Roche) and EnVision FLEX (Dako/Agilent)

Performance history
This was the fourth NordiQC assessment of BCL2. The pass rate increased compared to the previous run 57, 2019 (see Graph 1).

Graph 1. **Proportion of sufficient results for BCL2 in the four NordiQC runs performed**



Controls
Normal tonsil is recommended as positive and negative tissue control for demonstration of BCL2. Virtually all normal mantle zone B-cells and intra germinal centre T-cells of secondary follicles must show a moderate to strong, predominantly cytoplasmic staining reaction, whereas activated B-cells of the germinal centres must be negative. Due to variable occurrence of intact squamous epithelial in tonsillar tissue allowing the demonstration of weak to moderate expression for BCL2 in the basal epithelial cells, laboratories should consider including spleen as critical positive tissue control, as virtually all littoral cells of the sinusoids should show an at least weak staining intensity confirming the assays low limit of BCL2 detection.

Conclusion
The mAb clones **124**, **BCL-2/100/D5**, and the rmAb clone **SP66** are all robust antibodies for demonstration of BCL2. The rmAb clones **E17** and **EP36** can also be used but a significant proportion of protocols (42%) displayed an aberrant staining of nucleoli in several tissue cores - most predominant in the tonsil. For all assays applied in this assessment, the overall pass rate was 93% (406/437). 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. The Ready-to-Use (RTU) systems from the major vendors (**PA0117** Leica Biosystems**, IR614** Dako/Agilent**, 790-4464 and 790-4604** Ventana/Roche), and applying vendor recommended protocol settings, gave an overall high pass rate of 97% (95/98). Among these RTU systems, **PA0117** (Leica Biosystems) and **790-4604** (Ventana/Roche), based on mAb clones **BCL-2/100/D5** and the rmAb clone **SP66**, respectively, provided superior performance with 100% (45/45) sufficient results - 91% (41/45) being optimal.

Table 1a. **Overall results for BCL2, run 73**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	95	61	27	6	1	93%	64%
Ready-To-Use antibodies	342	244	74	22	2	93%	71%
Total	437	305	101	28	3		
Proportion		70%	23%	6%	1%	93%	

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

Table 1b. **Concentrated antibodies and assessment marks for BCL2, run 73**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **124**	63 6 1	Dako/Agilent Cell Marque Zytomed Systems	48	19	3	0	96%	69%
mAb clone **BCL-2/100/D5**	12	Leica Biosystems	8	2	2	0	83%	67%
mAb clone **100/D5**	1	Epredia	0	0	0	1	-	-
rmAb clone **E17**	3 2	Cell Marque Abcam	1	4	0	0	100%	20%
rmAb clone **SP66**	3 1	Cell marque Abcam	2	1	1	0	-	-
rmAb clone **QR062**	1	Quartett	0	1	0	0	-	-
rmAb clone **EP36**	1	Diagnostic Biosystems	1	0	0	0	-	-
rmAb clone **ZR130**	1	Zeta Corporation	1	0	0	0	-	-
Total	95		61	27	6	1	-	
Proportion			64%	29%	6%	1%	93%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for BCL2, run 73**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BCL-2/100/D5 PA0117**[3]	30	Leica Biosystems	27	3	0	0	100%	90%
mAb clone **BCL-2/100/D5 PA0117**[4]	11	Leica Biosystems	7	3	1	0	91%	64%
mAb clone **100/D5 PM003**	1	Biocare Medical	0	1	0	0	-	-
mAb clone **124 IR614**[3]	16	Dako/Agilent	13	2	1	0	94%	81%
mAb clone **124 IR614**[4]	89	Dako/Agilent	59	16	14	0	84%	66%
mAb clone **124 790-4464**[3]	37	Ventana/Roche	18	17	2	0	95%	49%
mAb clone **124 790-4464**[4]	65	Ventana/Roche	50	13	1	1	97%	77%
mAb clone **124 226M-XX**	3	Cell Marque	1	2	0	0	-	-
mAb clone **124 PDM016**	2	Diagnostic Biosystems	1	0	0	1	-	-
mAb clone **124 GM088702**	1	Gene Tech	1	0	0	0	-	-
mAb clone **BCL-2/100 AM287**	1	BioGenex	0	0	1	0	-	-
mAb clone **MX022 MAB-0711**	1	Fuzhou Maixin	1	0	0	0	-	-
mAb clone **C7B8 CBM-0041**	1	Celvnovte Biotechnology	1	0	0	0	-	-
rmAb clone **SP66 790-4604**[3]	15	Ventana/Roche	14	1	0	0	100%	93%
rmAb clone **SP66 790-4604**[4]	57	Ventana/Roche	44	13	0	0	100%	77%
rmAb clone **SP66 226R-XX**	2	Cell Marque	2	0	0	0	-	-
rmAb clone **EP36 8459-C010**	4	Sakura Fine Tech	2	1	1	0	-	-
rmAb clone **EP36 MAD-000675QD**	2	Master Diagnostica	1	1	0	0	-	-
Ab clone **8C8 I11642E-02**	1	Biolynx Biotechnology	0	1	0	0	-	-
Ab clone **361F0D1 PA545**	1	Abcarta/Abcepta	1	0	0	0	-	-
Ab clone **DY49015 4911822**	1	Dakewe Bio.	0	0	1	0		
rmAb clone **BY124 BFM-0113**	1	Bioin Biotechnology	1	0	0	0	-	-
Total	342		244	74	22	2		
Proportion			71%	22%	6%	1%	93%	

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 BCL2, Run 73
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **124**: Protocols with optimal results were all based on Heat Induced Epitope Retrievel (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (19/22)*, TRS pH 6 (3-in-1) (Dako/Agilent) (1/1), Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (3/5), Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (2/4), PRIME Epitope Retrieval Solution 1 (PERS1; Leica Biosystems) (1/1) and Cell Conditioning 1 (CC1; Ventana/Roche) (22/35) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 63 of 66 (95%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **BCL-2/100/D5**: Protocols with optimal results were all based on HIER in an alkaline buffer using TRS pH 9 (3-in-1) (Dako/Agilent) (2/2), BERS2 (Leica Biosystems) (3/3), PERS2 (Leica Biosystems) (2/2) and CC1 (Ventana/Roche) (1/2) 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, 7 of 7 (100%) laboratories produced a sufficient staining result (all assessed as optimal).

rmAb clone **SP66**: The two protocols with an optimal result were based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) as retrieval buffer. The rmAb was diluted in the range 1:300-500 and EnVision Flex+ (Dako/Agilent, GV800+GV809) was used as detection system.

rmAb clone **E17**: The protocol with an optimal result was based on HIER using BERS2 (Leica Biosystems) as retrieval buffer. The rmAb was diluted 1:100 and Bond Refine (Leica Biosystems, DS9800) was used as detection system.

rmAb clone **EP36**: The protocol with an optimal result was based on HIER using CC1 (Ventana/Roche) as retrieval buffer. The rmAb was diluted 1:30 and UltraView (Ventana/Roche, 760-500) was used as detection system.

rmAb clone **ZR130**: The protocol with an optimal result was based on HIER using BERS2 (Leica Biosystems) as retrieval buffer. The rmAb was diluted 1:300 and Bond Refine (Leica Biosystems, DS9800) was used as detection system.

Table 2. **Proportion of optimal results for BCL2 for the most commonly used antibody concentrates 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 pH6.1	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 **124**	4/4**	-	15/18 (83%)	1/1	21/34 (62%)	-	2/4	3/5 (60%)
mAb clone **BCL2/100/D5**	-	-	2/2	-	1/1	-	4/4	0/3

* 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, Ultra Plus
3) Bond III/MAX/PRIME

Ready-To-Use antibodies and corresponding systems (≥5 protocols).

mAb clone **BCL-2/100/D5**, product no. **PA0117**, Leica Biosystems, Bond III/MAX/PRIME:
Protocols with optimal results were typically based on HIER using BERS2 or PERS2 (efficient heating time 20 min. at 95-100°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 28 of 28 (100%) laboratories produced a sufficient staining result – 89% (25/28) being optimal.

mAb clone **124**, product no. **IR614**, 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 95-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000) or EnVision FLEX+ (K8000+K8021) as detection systems. Using these protocol settings, 19 of 21 (90%) laboratories produced a sufficient staining result – 67% (14/21) being optimal.

mAb clone **124**, product no. **790-4464**, Ventana/Roche, BenchMark Ultra/Ultra PLUS/XT:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 16-32 min. incubation of the primary Ab and OptiView (760-700) or UltraView with or without amplification (760-500+760-080) as detection system. Using these protocol settings, 76 of 79 (96%) laboratories produced a sufficient staining result – 68% (54/79) being optimal.

rmAb clone **SP66**, product no. **790-4604**, Ventana/Roche, BenchMark Ultra/Ultra PLUS/GX:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 48-64 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, 40 of 40 (100%) laboratories produced a sufficient staining result – 83% (33/40) 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 BCL2 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb 124 **IR614**	94% (15/16)	81% (13/16)	80% (8/10)	50% (5/10)
Leica BOND III/MAX/PRIME mAb BCL-2/100/D5 **PA0117**	100% (30/30)	90% (27/30)	91% (10/11)	64% (7/11)
VMS Ultra/Ultra PLUS/XT mAb 124 **790-4464**	95% (35/37)	49% (18/37)	97% (63/65)	77% (50/65)
VMS Ultra/Ultra PLUS rmAb SP66 **790-4604**	100% (15/15)	93% (14/15)	100% (56/56)	77% (43/56)

* 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 concordance with the previous assessments for BCL2, the prevalent feature of an insufficient result was characterized by a too weak or false negative staining result accounting for 90% (28/31) of all insufficient results. In the remaining 10% (3/31) of insufficient results, excessive background reactions were often seen in combination with uneven and/or an aberrant nuclear staining - compromising interpretation of the specific signal for BCL2.
Virtually all laboratories were able to demonstrate BCL2 in high-level antigen expressing cells, such as T-cells in the interfollicular zones and normal mantle zone B-cells of secondary follicles in the tonsil, whereas demonstration of BCL2 in intra-germinal centre T-cells of the tonsil, littoral cells lining sinusoids of the spleen and the neoplastic B-cells of the two follicular lymphomas was more challenging, requiring appropriate protocol settings for optimal performance.

As described in the previous report Run 57, 2019 for BCL2, follicular B-cell lymphomas show cytogenic abnormalities and is characterized by the translocation t(14;18)(q32;q21) resulting in constitutive overexpression of BCL2. However, a small number of cases show absence of BCL-2 expression despite displaying genetic aberrations/rearrangement. In these BCL2 IHC negative cases carrying rearrangement of the BCL2 gene locus, some somatic mutations of the translocated BCL2 gene might alter the immunohistochemical binding site, being unreactive with the standard mAb clones **124** and **100/D5** due to a shift/substitution of amino acid(s) in the region of the target epitope sequence. Yet, most of these IHC pseudo-negative follicular B-cell lymphomas are positive by immunohistochemistry using alternative clones as **SP66** and **E17** (alias EP36 – see previous report), references 1-4 below. In assessment run 57 for BCL2, we observed conflicting results as one follicular B-cell lymphoma in the NordiQC block (tested positive for rearrangement of the BCL2 gene by FISH) was negative for the rmAb clones **E17** and **EP36**, most likely due to "cryptic"/aberrant rearrangement and/or mutations of the translocated BCL2 gene locus impacting epitope binding site for **E17/EP36**, whereas the mAb clone **124** and rmAb clone **SP66** were positive in the same case.

In this assessment (run 73), and independent of antibody clone applied, the two follicular B-cell lymphomas both stained as expected for BCL2. In total, twelve protocols were based on the rmAb clones **E17** and **EP36**, both as concentrates and RTU formats, giving an overall pass rate of 92% (11/12) – 42% (5/12) being optimal. An aberrant staining reaction of nucleoli was seen in 42% (5/12) of the protocols and this deviating reaction pattern was accepted, as long as interpretation of the specific signals for BCL2 was not compromised - see Figs. 5a-5b.

Used as concentrate within a laboratory developed assay (LD), the mAb clone **124** was the most widely used antibody for demonstration of BCL2, providing a high pass rate of 96% (67/70) – 69% (48/70) being optimal (see Table 1b). Performance characteristic on the respective automatic platforms from the major vendors are outlined in Table 2, and as shown for the mAb clone **124**, optimal results could be obtained on all main instruments. The clone was most successful on Autostainer and Omnis providing the highest proportion of optimal results when applied in the "recommended/optimal" dilution range 1:10-200. The main prerequisites for an optimal staining result were use of efficient HIER in an alkaline buffer and a 3-layer detection system. Within LD-assays, three protocols based on the mAb clone **124**, were assessed as insufficient with a too weak staining reaction caused by technical parameters providing too low analytical sensitivity. One protocol was based on less efficient HIER in acidic buffer in combination with too diluted primary antibody and two protocols were based on the less sensitive 2-step detection systems (UltraView and EnVision FLEX).

The mAb clone **BCL-2/100/D5** used as concentrate within LD-assay, gave a pass rate of 83% (10/12) – 67% (8/12) being optimal. Optimal results could be obtained on all fully automated platforms (see Table 2). All optimal results (7/7) were based on protocol settings based on efficient HIER in an alkaline buffer, the average dilution factor was 1:64 (range 1:25-100) and 3-step detection systems as Bond Refine, OptiView or Envision FLEX+ were applied. Four protocols were downgraded and gave a result scored as either" good" or "borderline" (see Table 1b), likely related to the use of the less efficient HIER buffer BERS1 (3 protocols) and too diluted antibody (1 protocol).

78% (342/437) of all laboratories used an RTU format for the demonstration of BCL2. This is an increase compared to the former run 57, 2019 in which 63% (200/319) of the participants applied a RTU format. In this assessment, and using protocols in compliance with the vendor recommended protocol settings (VRPS), the RTU systems **PA0117** (Leica Biosystem/Bond platforms), **IR614** (Dako/Agilent Autostainer), **790-4464** (Ventana/BenchMark platforms) and **790-4604** (Ventana/BenchMark platforms) based on the mAb clones **BCL-2/100/D5,** (Leica Biosystems), **124** (both Dako/Agilent and Ventana/Roche) and the rmAb clone **SP66** (Ventana/Roche), respectively, were all successful for demonstration of BCL2 (see Table 3). Taken together, the pass rate was 97% (95/98) of which 73% (72/98) were assessed as optimal – highest proportion of optimal results was obtained with the RTU format **PA0117** (Leica Biosystems) and **790-4604** (Ventana/Roche) (see Table 3). These RTU systems are very robust and applying laboratory modified protocol settings (LMPS) did not improve the staining quality except for the RTU system **790-4464** Ventana/Roche (see Table 3), giving significantly higher proportion of optimal results compared to protocols based on VRPS, typically applying a 3-step multimer detection system e.g., UltraView with amplification and/or prolonging incubation time in the primary antibody. In total and using these systems on the intended stainer device but with modified protocol settings, eight protocols were assessed as insufficient - primarily related to the use of less sensitive detection systems EnVision Flex and UltraView (7 protocols) or applying HIER in the less efficient low pH buffer BERS1 (1 protocol).

Numerous laboratories used a RTU format off-label on non-validated platforms giving an overall pass rate of 85% (68/80). A significant proportion of participants were using the RTU product **IR614** (developed and validated for the Autostainer) based on the mAb clone **124** on the Omnis platform, giving a pass rate of 84% (57/68) – 74% (50/68) being optimal. Optimal performance was typically based on HIER in TRS High pH, incubation time in primary Ab in the range 15-30 minutes and use of EnVision Flex+ as the detection system. These protocol settings were applied by 94% (47/50) of the participants giving an optimal mark. In comparison and using the same protocol settings as above except for substituting EnVision Flex+ with Envision Flex, all (11/11) protocols were assessed as insufficient ("borderline"). In general, the use of a RTU product off-label is not recommendable, unless each laboratory has performed an extensive and thorough validation process, documenting the accuracy and analytical sensitivity/specificity of the test. However, and in the light of an absent true validated RTU system for BCL2 developed for the Dako Omnis platform, laboratories are forced to use alternatives e.g., the RTU product **IR614**. In this assessment a successful mitigation of the **IR614** was observed when using this product, typically with HIER in TRS High pH for 30 minutes, primary Ab incubation for 20 min and EnVision Flex+ as detection system.

The Ventana Benchmark users could select from two RTU products from the same vendor Ventana/Roche, **790-4464** or **790-4604**, based on the mAb clone **124** and **SP66**, respectively. In total, 40% (174/437) of all protocols were based on these two RTU systems. As shown in Table 3, and focusing on VRPS, both systems gave a high proportion of sufficient results, but the RTU system **790-4604** provided significantly higher proportion of optimal results compared to the RTU system **790-4464**, 93% and 49%, respectively. The RTU system **790-4604**, seems very robust for demonstration of BCL2 and several parameters could be used to obtain an optimal result including use of the less sensitive detection system UltraView and thus, if laboratories favor use of this detection system, the RTU system **790-4604** based on the rmAb clone **SP66** is preferable to the RTU system **790-4464** based on the mAb clone **124**.

This was the fourth assessment of BCL2 in NordiQC (see Graph 1). The pass rate increased slightly to 93% in this assessment compared to 89% in the previous run 57 (2019). In this assessment, the most common cause for an insufficient staining result was related to the use of less efficient HIER in an acidic buffer e.g. BERS1 or application of the less sensitive detection systems EnVision Flex or UltraView often in combination with the use of the mAb clone **124**, accounting for 77% (24/31) of all insufficient results - typically giving a too weak staining reaction of critical staining indicators as e.g., littoral cells of the spleen. Importantly, protocol settings must be carefully calibrated according to the expected reaction patterns of the recommended positive and negative control materials.

References;

1. Maeshima AM, Taniguchi H, Furukawa H, et al. Diagnostic clues of BCL2-negative, faint, or controversial follicular lymphomas: a study of 103 cases. Hum Pathol. 2023 May;135:84-92. Doi: 10.1016.
2. Schraders, M., de Jong, D., Kluin, P et al. Lack of Bcl-2 expression in follicular lymphoma may be caused by mutations in the *BCL2* gene or by absence of the t(14;18) translocation. J. Pathol., 2005: 329-335. Doi:10.1002.
3. Patrick Adam, Rosalie Baumann, Janine Schmidt et al. The BCL2 E17 and SP66 antibodies discriminate 2 immunophenotypically and genetically distinct subgroups of conventionally BCL2-"negative" grade 1/2 follicular lymphomas. Human Pathol. 2013 44: 1817-1826. Doi:10.1016
4. Noraidah Masir, Lisa J. Campbell, Margaret Jones, David Y. Mason. Pseudonegative BCL2 protein expression in a t(14;18) translocation positive lymphoma cell line: a need for an alternative BCL2 antibody. Pathology, 2010. 42;3: 212-216. Doi 10.3109.

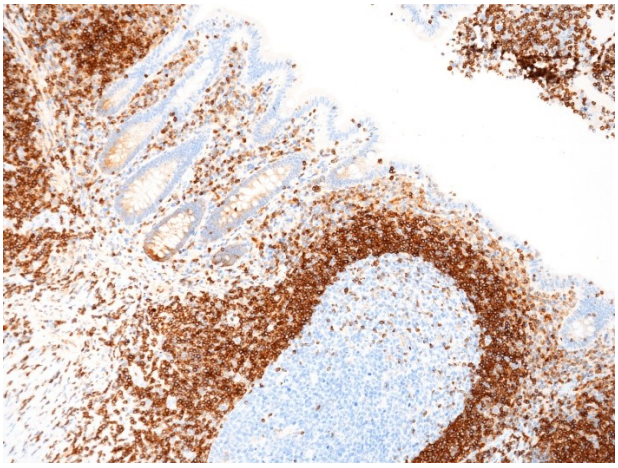


Fig. 1a (x100)
Optimal BCL2 staining reaction of the appendix using the RTU format (Dako/Agilent, **IR614**) based on mAb clone **124** off-label on the Omnis instrument, largely following recommendations given by the vendor for the Autostainer except for substituting EnVision Flex with EnVision Flex+ (with mouse linker) – same protocol used in Figs. 1a – 4a.
Virtually all mantle zone B-cells and intra-germinal centre T-cells of secondary follicles show a moderate to strong, distinct cytoplasmic staining reaction. Activated intra-germinal centre B-cells and luminal epithelial cells are negative as expected.

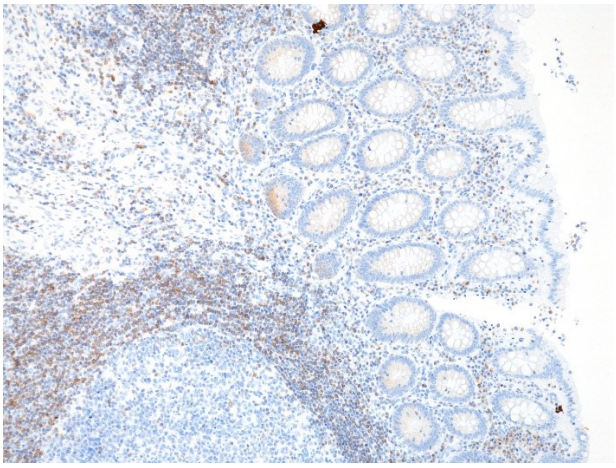


Fig. 1b (x100)
BCL2 staining of the appendix using the same RTU format off-label on the Omnis platform as in Fig. 1a, primarily following vendor recommended protocol settings to the Autostainer using Envision Flex (without mouse linker) as the detection system – same protocol used in Figs. 1b – 4b.
The protocol provided too low analytical sensitivity, and both mantle zone B-cells and intra-germinal centre T-cells displayed too weak staining intensity. In general, this direct method transfers of a RTU format validated to a specific platform to another non-validated platform is not recommendable and, in this case, gave too weak staining intensity. Importantly, and using a RTU product on a non-validated platform, laboratories must perform a full technical validation to ensure accuracy and precision of the IHC test.

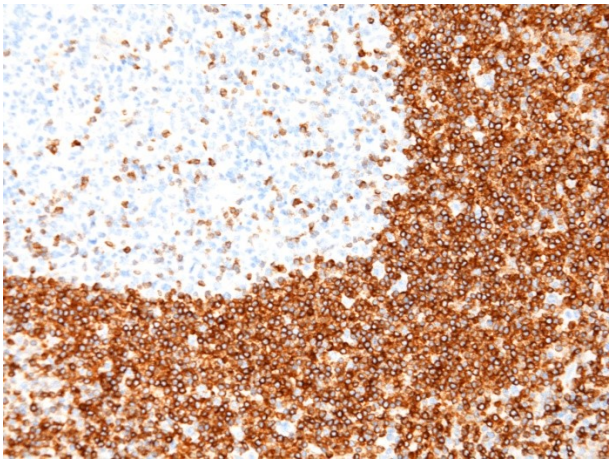


Fig. 2a (x200)
Optimal BCL2 staining reaction of the tonsil using same protocol as in Fig. 1a. Virtually all mantle zone B-cells and intra-germinal centre T-cells of secondary follicles display a moderate to strong distinct cytoplasmic staining reaction, whereas activated B-cells of the germinal centre are negative as expected.

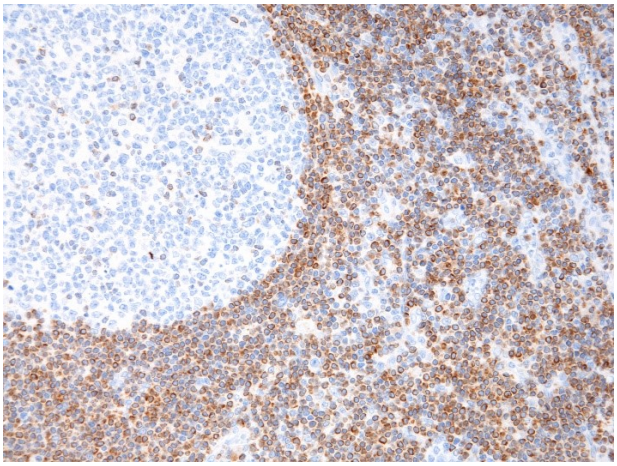


Fig. 2b (x200)
BCL2 staining reaction of the tonsil using same protocol as in Fig. 1b. The staining intensity is too weak, and the intra-germinal centre T-cells display only a faint to weak staining reaction or are complete false negative – compare with the protocol in Fig 2a.

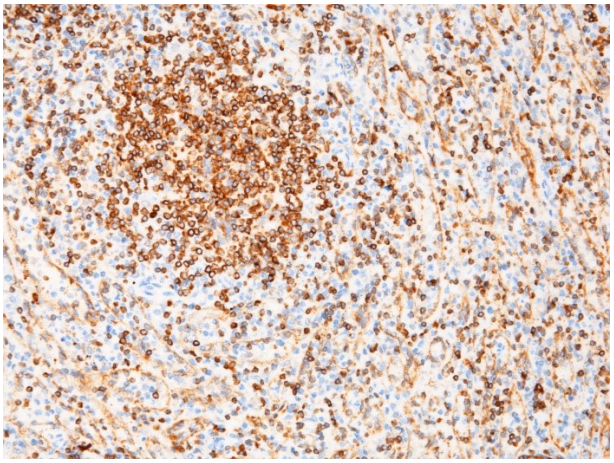


Fig. 3a (x200)
Optimal BCL2 staining reaction of the spleen using same protocol as in Figs. 1a - 2a. All littoral cells lining the sinusoids (red pulp) display a weak to moderate and distinct staining reaction, whereas lymphatic cells of the white pulp are strongly demonstrated.

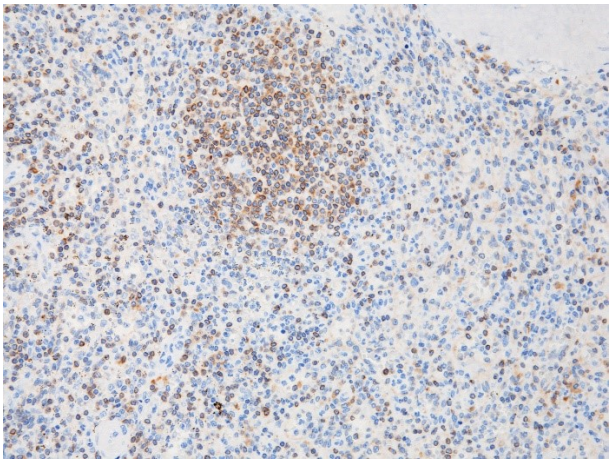


Fig. 3b (x200)
BCL2 staining reaction of the spleen using the same protocol as in Figs. 1b - 2b. Virtually all littoral cells of the red pulp are false negative, and the lymphatic cells of the white pulp display a too weak staining intensity - compare with Fig. 3 a.
Spleen is a promising tissue control for low level detection of BCL2 and laboratories should consider, especially in the validation process but also as daily control for BCL2, to include normal spleen to monitor precision of the IHC assay with focus on low limit of demonstration - see expected reaction pattern in Fig. 3a.

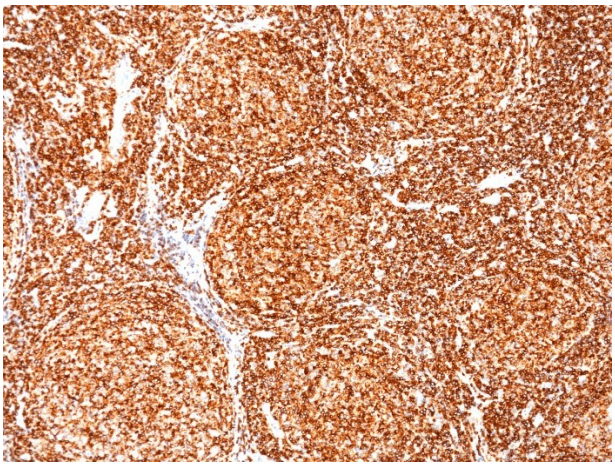


Fig. 4a (x100)
Optimal BCL2 staining reaction of the follicular B-cell lymphoma (tissue core no. 5) using same protocol as in Figs. 1a - 3a. All neoplastic B-cells are strongly positive.

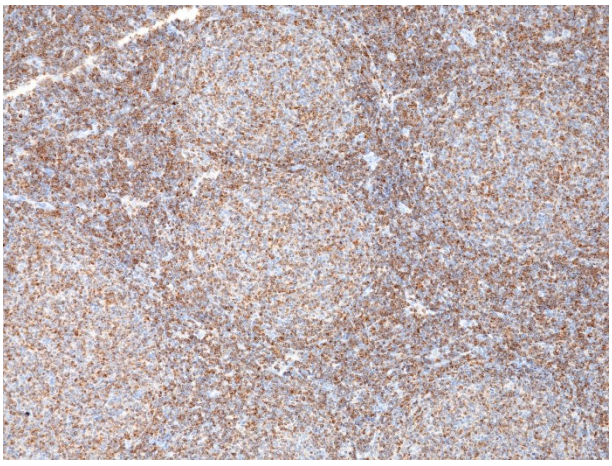


Fig. 4b (x100)
BCL2 staining reaction of the follicular B-cell lymphoma (tissue core no. 5) using same protocol as in Figs. 1b - 3b. The staining intensity and proportion of positive neoplastic B-cells are significantly reduced - compare with Fig. 4a.
Although the staining is acceptable for clinical purpose, the protocol provided too low analytical sensitivity, and thus, risking misclassification of lymphomas displaying weaker levels of expression for BCL2. Particularly, discrimination between reactive (hyperplastic lymph node) versus neoplastic follicles of follicular B-cell lymphomas might be challenging.

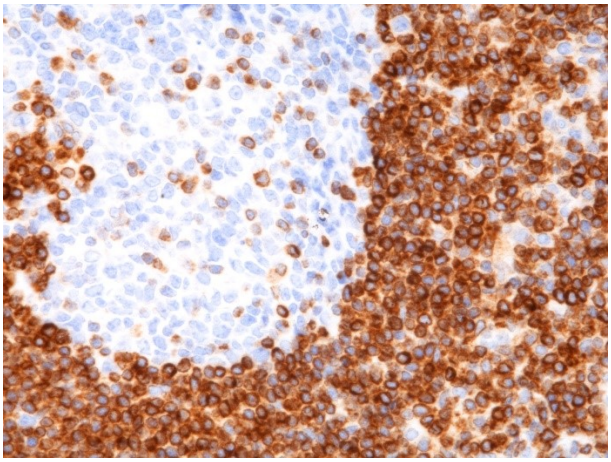


Fig. 5a (x400)
Optimal BCL2 staining reaction of the tonsil using the RTU system **8459-C010** based on rmAb clone **EP36 (same as E17)** (Sakura Fine Tek, lot. 8459B09624) on the Sakura Tissue-Tek Genie instrument. The staining gave the expected reaction pattern, and no aberrant staining of nucleoli could be seen.

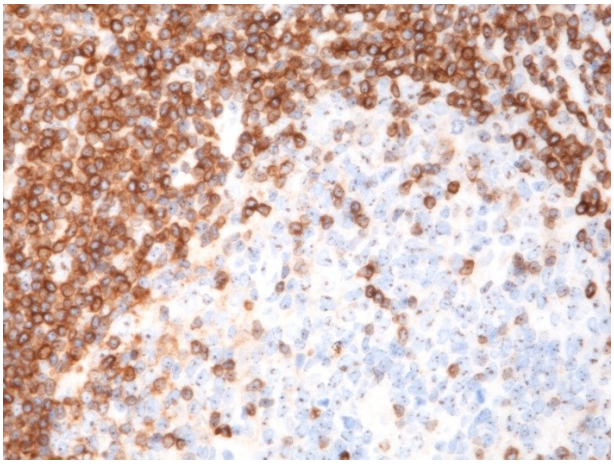


Fig. 5b (x400)
BCL2 staining of the tonsil using exactly same protocol settings on the same platform as in Fig. 5a except for applying a different batch number of the primary antibody (Sakura Fine Tek, lot. 8459A02924). The protocol was assessed as optimal, but a distinct deviating staining of nucleoli was observed (accepted as it did not compromise interpretation of the specific signals for BCL2). The explanation for this aberrant staining pattern of nucleoli is difficult to elucidate upon as only few data points are available for assessment, but in this case, the only difference between the two protocols in Figs. 5a – 5b, is the lot number applied.

MB/LE/SN 25.03.2025