Assessment Run 70 2024

Bcl-6 protein (BCL6)

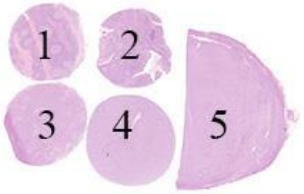
Purpose

Evaluation of the technical performance, and in particular the level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for BCL6, primarily used for subclassification of B-lymphomas and to discriminate Diffuse Large B-Cell Lymphoma (DLBCL) of germinal center B-cell like (GCB) from non-germinal center/activated B-cell (non-GCB/ABC) subtype. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for BCL6 (see below). Cases diagnosed with DLBCL were classified according to Hans[1] algorithm in which neoplastic B-cells of the GCB phenotype is characterized being CD10 positive or BCL6 positive in absence of CD10 and MUM1. A cut-off value of ≥30% positive neoplastic B-cells was applied.

[1]*Hans CP, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275-82.*

Material

The slide to be stained for BCL6 comprised:
1-2. Tonsils, 3. DLBCL (GCB subtype), 4. DLBCL (non-GCB/ABC subtype), 5. Follicular lymphoma (FL)



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Bcl-6 staining as optimal included:

- A moderate to strong, distinct nuclear staining reaction of virtually all germinal centre B-cells in the tonsils.
- An at least weak to moderate, distinct nuclear staining reaction of the majority of the squamous epithelial cells in the tonsils.
- A strong, distinct nuclear staining reaction of all the neoplastic B-cells in the follicular lymphoma.
- An at least moderate, distinct nuclear staining reaction of virtually all the neoplastic cells in the DLBCL (GCB subtype).
- No staining reaction of or explicitly below 30% positive neoplastic cells in the DLBCL (non-GCB/ABC subtype). Dispersed normal B-cells intermingling between the neoplastic B-cells should be distinctively demonstrated.
- In the tonsils, the majority of lymphocytes (e.g., T-cells) should be negative including mantle zone B-cells of primary follicles in which only a minor subpopulation should be weakly stained.

KEY POINTS FOR BCL6 IMMONUASSAYS

- The mAb clones **GI191E/A8**, **LN22** and **PG-B6p** are recommendable Abs.
- RTUs gave in general a lower pass rate compared to concentrates and requires optimization.
- Efficient HIER should be performed in an alkaline buffer.
- Incubation time of primary Ab should not be in too short time.

Participation

Number of laboratories registered for BCL6, run 70	401
Number of laboratories returning slides	373 (93%)

All slides returned after the assessment were assessed and received advice if the result being insufficient, but the data were not included in this report.

Results

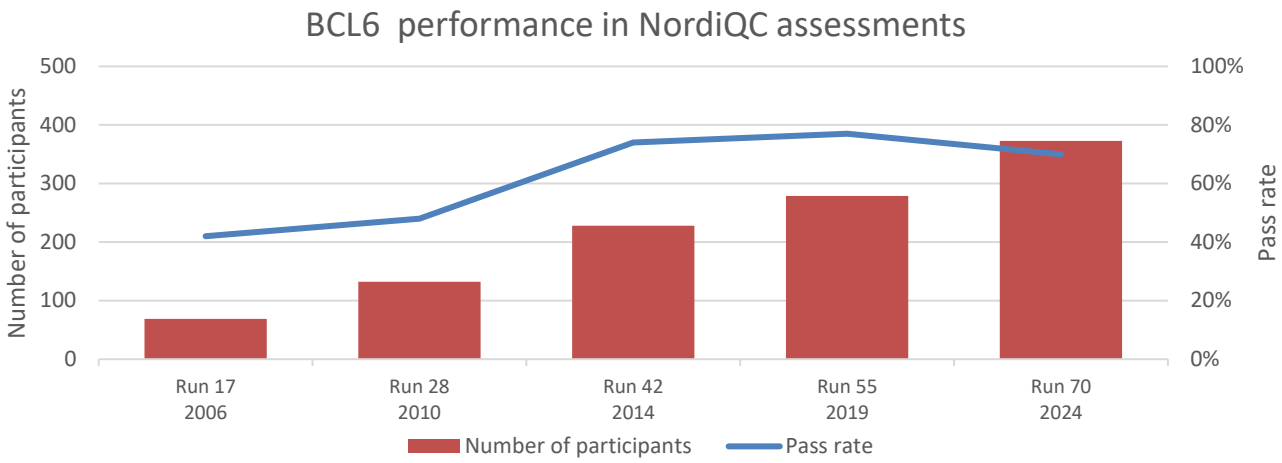
373 laboratories participated in this assessment and 261 (70%) achieved a sufficient mark (optimal or good), see Table 1a (see page 2). Table 1b and 1c summarizes antibodies (Abs) used and assessment marks (see page 3).

Accredited by DANAK under registration number 616 to proficiency testing.

The most frequent causes of insufficient staining reactions were:
- Insufficient Heat Induced Epitope Retrieval (HIER) (too short HIER time)
- Too short incubation time in primary antibody
- Less successful performance of RTU products based on the mAb clone GI191E/A8
- Unexplained technical issues

Performance history
This was the fifth NordiQC assessment of BCL6. The pass rate decreased compared to the previous run 55, 2019 (see Graph 1).

Graph 1. **Proportion of sufficient results for BCL6 in the five NordiQC runs performed**



Controls
Normal tonsil is recommended as positive and negative tissue control. Virtually all the germinal centre B-cells must show a moderate to strong nuclear staining reaction, while an at least weak to moderate nuclear staining reaction must be seen in the majority of squamous epithelial cells. In the mantle zones and interfollicular areas only dispersed cells should display a positive nuclear staining reaction.

Conclusion
The mAbs clones **GI191E/A8**, **LN22**, **PG-B6p** and the rmAb clones **E5I8I, EP278, DA005** could all be used to produce optimal staining results for BCL6. Irrespective of the clone applied, efficient HIER in alkaline buffer, use of a highly 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, the Dako/Agilent RTU product GA625 based on the mAb clone PG-B6p provided the highest proportion of sufficient and optimal results and especially if laboratory modified protocol settings were applied – primarily prolonging incubation time in primary antibody. Protocols based on the mAb clone GI191E/A8 (Cell Marque and Ventana/Roche) were challenged by poor signal-to-noise ratio e.g., excessive background staining and for the RTU formats, only 14% (22/162) were given an optimal mark.

Table 1a. **Overall results for BCL6, run 70**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	87	41	29	15	2	80%	47%
Ready-To-Use antibodies	286	89	102	86	9	67%	31%
Total	373	130	131	101	11		
Proportion		35%	35%	27%	3%	70%	

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

Table 1b. **Concentrated antibodies and assessment marks for BCL6, run 70**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **PG-B6p**	21	Dako/Agilent	8	9	3	1	81%	38%
mAb clone **LN22**	38 1 1	Leica Biosystems Epredia Diagnostik BioSystems	23	9	7	1	80%	58%
mAb clone **GI191E/A8**	22	Cell Marque	9	9	4	0	82%	41%
mAb clone **MX042**	1	Fuxhou Maixin	0	0	1	0	-	-
rmAb clone **E5I8I**	1	Cell Signaling	1	0	0	0	-	-
rmAb clone **QR047**	1	Quartett	0	1	0	0	-	-
rmAb clone **BP6191**	1	Biolynx Biotechnology	0	1	0	0	-	-
Total	87		41	29	15	2	-	
Proportion			47%	33%	17%	3%	80%	

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 BCL6, run 70**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **LN22 PA0204**[3]	23	Leica Biosystems	10	7	6	0	74%	43%
mAb clone L**N22 PA0204**[4]	16	Leica Biosystems	10	2	3	1	75%	63%
mAb clone **LN22 MAD-000638QD**	3	Master Diagnostica	1	1	0	1	-	-
mAb clone **PG-B6p IR625**[3]	8	Dako/Agilent	3	2	3	0	63%	38%
mAb clone **PG-B6p IR625**[4]	16	Dako/Agilent	6	1	8	1	44%	38%
mAb clone **PG-B6p GA625**[3]	36	Dako/Agilent	20	11	4	1	86%	56%
mAb clone **PG-B6p GA625**[4]	37	Dako/Agilent	24	10	3	0	92%	65%
mAb clone **GI191E/A8 760-4241**[3]	21	Ventana/Roche	0	16	5	0	76%	0%
mAb clone **GI191E/A8 760-4241**[4]	98	Ventana/Roche	10	42	42	4	53%	10%
mAb clone **GI191E/A8 227M-9x**	21	Cell Marque	3	10	7	1	62%	14%
mAb clone **MX042 MAB-0746**	1	Fuzhou Maixin	0	0	1	0	-	-
rmAb clone **EP278 8461-C010**	2	Sakura FineTek	1	0	1	0	-	-
rmAb clone **EP278 227R-28**	1	Cell marque	0	0	1	0	-	-
rmAb clone **EP278 PR166**	1	Path N Situ	0	0	1	0	-	-
rmAb clone **DA005 RMB1A051**	1	Dartmon Biotechnology	1	0	0	0	-	-
rmAb clone **907E3E6 PA155**	1	Abcarta	0	0	1	0	-	-
Total	286		89	102	86	9		
Proportion			31%	36%	30%	3%	67%	

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 BCL6, Run 70
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **PG-B6p**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (5/9)*, Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (1/3) and Cell Conditioning 1 (CC1; Ventana/Roche) (2/9) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:35 depending on the total sensitivity of the protocol employed. Using these protocol settings, 14 of 16 (88%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **LN22**: Protocols with optimal results were all based on HIER using CC1 (9/16), TRS pH 9 (3-in-1) (3/5), BERS2 (8/15), TRIS-EDTA/EGTA pH 9 (2/2) and Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:40-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 27 of 32 (84%) laboratories produced a sufficient staining result.

mAb clone **GI191E/A8**: Protocols with optimal results were all based on HIER using CC1 (7/16) and TRS pH 9 (3-in-1) (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:100-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 13 of 14 (93%) laboratories produced a sufficient staining result.

rmAb clone **E5I8I**: Protocol with an optimal result was based on HIER buffer H (Epredia) as retrieval buffer. The rmAb was diluted 1:50 and Envision Flex with Dual linkers (Dako/Agilent, GV800/823 + GV809/821) was used as detection system (FLEX++).

Table 2. **Proportion of optimal results for BCL6 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 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 **PG-B6p**	2/3**	-	3/5 (60%)	-	1/7 (14%)	-	1/1	-
mAb clone **LN22**	-	-	2/3	-	9/14 (60%)	-	7/11 (63%)	1/1
mAb clone **GI191E/A8**	1/1	-	1/1	-	6/12 (50%)	-	0/1	-

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

Ready-To-Use antibodies and corresponding systems
mAb clone **LN22**, product no. **PA0204**, Leica Biosystems, Bond-MAX/III/PRIME:
Protocols with optimal results were based on HIER using BERS2 (efficient heating time 20-40 min. at 100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 27 of 34 (79%) laboratories produced a sufficient staining result.

mAb clone **PG-B6p**, product no. **IR625**, 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+K8021) as detection systems. Using these protocol settings, 8 of 14 (57%) laboratories produced a sufficient staining result.

mAb clone **PG-B6p**, product no. **GA625**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 30 min. at 97°C), 12.5-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800/823+GV821) as detection systems. Using these protocol settings, 55 of 62 (89%) laboratories produced a sufficient staining result.

mAb clone **GI191E/A8**, product no. **760-4241**, Ventana/Roche, BenchMark Ultra/Ultra PLUS/XT/GX:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 8-32 min. incubation of the primary Ab and OptiView (760-700) as detection system. Using these protocol settings, 30 of 51 (59%) laboratories produced a sufficient staining result.

rmAb clone **EP278**, product no. **8461-C010**, Sakura Finetek, Tissue-Tek Genie Advanced stainer:
One protocol with an optimal result was based on HIER using Tissue-Tek Genie High pH Antigen Retrieval (efficient heating time 60 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit, DAB (8826-K250) as detection system.

mAb clone **DA005**, product no. **RMB1A051**, Shenzhen D. Biotechnology, AS330PLUS-Stainer:
One protocol with an optimal result was based on HIER using Dartmon Antigen Retrieval Buffer (efficient heating time 15 min. at 100°C), 30 min. incubation of the primary Ab. and Dartmon Immunochromogenic Reagent (DMRD4044) as detection system.

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 BCL6 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb PG-B6p **IR625**	63% (5/8)	38% (3/8)	47% (7/15)	40% (6/15)
Dako Omnis mAb PG-B6p **GA625**	86% (31/36)	56% (20/36)	94% (33/35)	69% (24/35)
Leica BOND MAX/ III/PRIME mAb LN22 **PA0204**	74% (17/23)	43% (10/23)	77% (10/13)	62% (8/13)
VMS Ultra/PLUS/XT/GX mAb GI191E/A8 **760-4241**	76% (16/21)	0% (0/21)	53% (52/98)	10% (10/98)

* 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 fifth assessment of BCL6, the prevalent features of an insufficient staining reaction were related to poor signal-to-noise ratio (excessive background staining/granular staining reaction due to use of amplification with tyramide (Ventana/Roche BenchMark systems), increased number and/or false positive staining of cells expected to be negative e.g., mantle zone B-lymphocytes of primary follicles in the tonsils. This was observed in 61% (68 /112) of the insufficient results and as described in the previous report (Run 55, 2019) for BCL6, primarily correlated to use of concentrates and RTU formats based on the mAb clone **GI191E/A8**.
In the remaining insufficient protocols (39%, 44/112), a too weak or false negative staining reaction was seen often in combination with an uneven staining reaction, impaired morphology and excessive counter/background staining - compromising interpretation of the specific signal for BCL6.
Virtually all laboratories were able to demonstrate BCL6 in high-level antigen expressing cells, such as normal germinal centre B-cells in the tonsils and the neoplastic cells of the follicular lymphoma. However, demonstration of BCL6 in low-level antigen expressing cells as squamous epithelial cells of the tonsil, the neoplastic cells of DLBCL (GCB type), but also of the DLBCL (non-GCB/ABC type) in which demonstration of dispersed normal B-cells intermingling between the negative neoplastic B-cells was more challenging, requiring a meticulous calibrated protocol avoiding an assay giving too low analytical sensitivity or reduced specificity.

Used within laboratory developed assays (LD) or Ready-to-Use formats (RTU), the mAb clones **LN22**, **PG-B6p** and **GI191/A8** were the most widely used antibodies for demonstration of BCL6 – being applied by 97% (362/373) of the laboratories. Using these antibodies within LD-assays (concentrated formats), the pass rate and proportion of optimal results were relatively low (see Table 1a and 1b). Performance characteristic of the individual concentrated formats applied on the respective automatic platforms from different vendors are outlined in Table 2. Overall, and for these commonly used concentrated formats, virtually all protocols assessed as optimal were based on efficient HIER in an alkaline buffer (39/40) and in addition the primary antibodies were carefully calibrated as described above (see page 4/Concentrated antibodies) and a sensitive 3-step detection system was applied (38/40) e.g., EnVision Flex+ (Dako/Agilent), Bond refine (Leica Biosystems) or OptiView (Ventana/Roche). In general, no single or specific protocol parameters could be identified unraveling the sources for the less successful performance. However, and for the concentrated format mAb clone GI191/A8, it was observed that 45% (10/22) of the

protocols gave excessive background staining reactions of which 40% (4/10) were assessed as insufficient. In this context, at present no specific lot numbers could be identified discriminating less successful from successful performance. 82% (18/22) of the participants used this format/concentrate on the Benchmark platforms (Ventana/Roche) and protocols were all based on OptiView as detections system of which only 39% (7/18) were giving an optimal mark. As the proportion of optimal results was low, protocol optimization seems to be quite challenging from a technical point of view, requiring that all technical parameters (excluding OptiView as the detection system) are carefully selected and precisely calibrated.

77% (286/373) of the laboratories used an RTU format for demonstration of BCL6. This is a significant increase compared to the former run 55, 2019 in which 64% (178/279) of the participants applied a RTU format.

In this assessment, and using vendor recommended protocol settings (VRPS), the RTU system **PA0204** (Leica Biosystem/Bond platforms) based on the mAb clone **LN22**, gave 74% (17/23) and 43% (10/23) sufficient and optimal results, respectively (see Table 3). Applying the RTU system PA0204 with laboratory developed protocol settings (LMPS), the proportion of optimal results increased significantly to 62% (8/13) mainly due to prolonged HIER time and/or prolonged incubation time in primary antibody. Protocols assessed as insufficient, were either based on VRPS (6/9) (HIER in BERS2 for 20 min., 15 min. incubation time in primary antibody and Bond Refine as the detection system) or reduced HIER Time in BERS2 (3/9). These results points in opposite directions and are difficult to elucidate upon as a significant proportion of participants applying VRPS also obtained optimal results (43%, 10/23) and thus, needs to be addressed by the vendor in collaboration with the affected laboratories to unravel if any causes for these deviating results can be found. Questions related to lot variation of the RTU product could be an explanation for the divergent results, but due to the limited number of data point's pr. lot number used by the participants, it is difficult to draw any definitive conclusions from this assessment. Yet, it was observed that the lot 76184 (3 protocols) and 75264 (4 protocols) gave insufficient and optimal results, respectively.

The RTU system **IR625** (Dako/Agilent) based on the mAb clone **PG-B6p** and developed for the Dako Autostainer provided the lowest proportion of sufficient and optimal results among "plug-and-play" RTU systems, 63% (5/8) and 38% (3/8), respectively, even though protocol settings should provide a high and expected analytical sensitivity of the assays. It was observed that LMPS did not improve the performance, in contrary (see Table 3). However, the antigenic epitope to which the antibody clone PG-B6p is raised against, is sensitive to peroxidase blocking and this might partly explain the moderately poor performance of the RTU assay IR625. This observation has also been specified in previous runs for BCL6, e.g., run55, 2019. The antibody clone PG-B6p require that the endogenous peroxidase blocking step is performed after application of the primary Ab.

In comparison and using VRPS, the RTU system **GA625** (Dako/Agilent), also based on the mAb clone **PG-B6p** and developed for the Omnis platform provided the highest proportion of sufficient and optimal results among all other RTU systems from the major vendors, 86% (31/36) and 56% (20/36), respectively. Using LMPS an improved performance was seen which contrasted with the corresponding RTU product IR625 on the Autostainer (see Table 3). On the Omnis platform, the endogenous peroxidase blocking step is applied after application of the primary antibody with no or only minor effect of BCL6 antigenic epitopes, and in particular, facilitating the binding of the antibody clone PG-B6 to the retrieved BCL6 protein. However, the improved pass rate and proportion of optimal results gained using LMPS for the RTU system GA625, was primarily correlated to the usage of prolonged incubation time in the primary antibody; The vendor recommended incubation time in primary antibody is 12.5 min. whereas the average incubation time in primary antibody was 26 min. (range 12.5-45 min.) using modified protocol settings. For the protocols assessed as insufficient, no technical factors could be identified and thus, explain for the poor performance as these parameters were identical to protocols giving optimal results.

Applying VRPS, the RTU system from Ventana/Roche **760-4241** based on the mAb clone **GI191/A8** and developed for the Benchmark platforms, provided a pass rate of 76% (16/21) - none being optimal (see Table 3). As observed with the concentrated formats, the RTU format was also challenged due to poor signal-to-noise ratio and in fact, none (0/40) of the protocols (all settings) based on UltraView as detection system (vendor recommended) with or without amplification, could produce an optimal result. In these assays, 93% (37/40) of the protocols displayed an excessive background staining and often in combination with increased number of positive neoplastic B-cells in the DLBCL non-GCB/ABC type. Using LMPS, the pass rate decreased significantly to 53% (52/98) but 10% (10/98) were assessed as optimal - all 10% based on OptiView as the detection system. No technical parameters could be identified e.g., HIER time, incubation time in primary antibody and/or lot variations, unravelling why these protocols were giving an optimal result. Overall, and applying the protocol settings giving an optimal result - HIER in CC1 32-64 min., incubation time in primary antibody in the range 8-32 min and OptiView as detection system, the pass rate was only 60% (32/53). On par to UltraView as detection system, OptiView also displayed

problems with poor-signal-to noise ratio/excessive background staining - seen in 77% (41/53) of the protocols applying "optimal" settings as described above.
Eight laboratories used the RTU format with OptiView in combination with Tyramide amplification and none (0/8) were assessed as optimal, typically showing unspecific and an aberrant granular staining pattern.

Twenty-one laboratories used the RTU format **227M-90/97/98** (Cell marque) also based on the mAb clone **GI191/A8**. Virtually all laboratories (20/21) used this format on the Ventana Benchmark platforms, providing a pass rate of 65% (13/20) - 15% (3/20) being optimal. In general, 75% (15/20) of the protocols displayed the same problem as described above for the RTU system 760-4241 (Ventana/Roche) but also seen with the concentrated formats e.g., giving excessive background staining.

This was the fifth assessment of BCL6 in NordiQC (see Graph 1). The pass rate decreased to 70% in this assessment compared to 77% in the previous run 55 (2019). In this assessment, the most common cause for an insufficient staining result was related to the use of the mAb clone GI191/A8, accounting for 56% (63/112) of all insufficient results - typically giving poor signal-to-noise ratio. In total, 43% (162/373) of the participants used this antibody providing an overall pass rate of 61% (99/162) - only 14% (22/162) being optimal, and thus, participants and vendor(s) should consider substituting this demanding antibody with an alternative for the demonstration of BCL6. Importantly, all protocol settings must be carefully calibrated according to the expected reaction patterns of the recommended control material (see below).

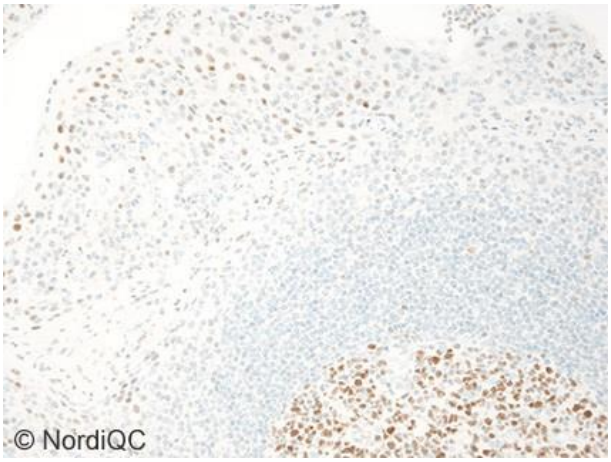


Fig. 1a (x200)
Optimal BCL6 staining reaction of the tonsil (tissue core no. 2) using the mAb clone LN22 as concentrate, optimally calibrated with HIER in CC1 (32 min. at 100°C) and OptiView as detection system – Same protocol used in Figs. 2a – 4a.
Virtually all germinal centre B-cells show a moderate to strong nuclear staining reaction and the majority of nuclei in the squamous epithelial cells (surface epithelium) display a weak to moderate reaction.

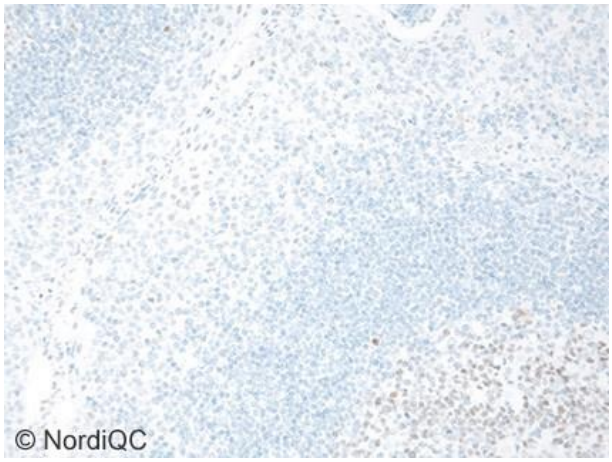


Fig. 1b (x200)
Insufficient BCL6 staining reaction of the tonsil (tissue core no. 2) using the same clone/format as in Fig. 1a, but with a protocol providing too low analytical sensitivity; too diluted primary antibody in combination with UltraView as the detection system – same protocol used in Figs. 2b – 4b.
Overall the staining intensity is significantly reduced and virtually all squamous epithelial cells are negative or only faintly demonstrated – same field as in Fig.1a.

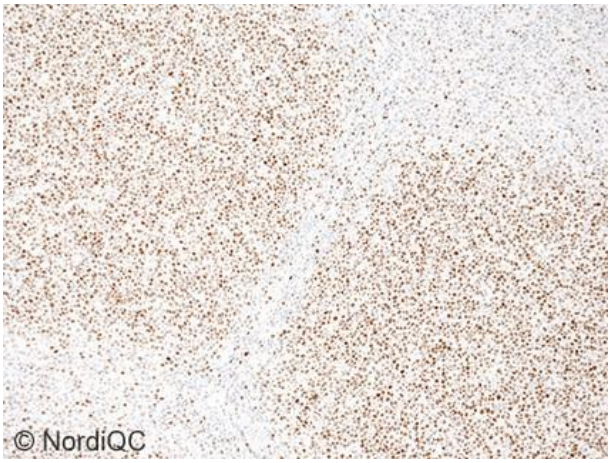


Fig. 2a (x100)
Optimal staining reaction for BCL6 of the follicular lymphoma using same protocol as in Fig. 1a. All neoplastic B-cells cells display a strong and distinct nuclear staining reaction.

Fig. 2b (x100)
Insufficient BCL6 staining reaction of the follicular lymphoma using same protocol as in Fig. 1b. Although the reaction pattern is similar to result obtained in Fig. 2a, the staining intensity is too weak and proportion of positive neoplastic B-cells is reduced – same field as in Fig. 2a.

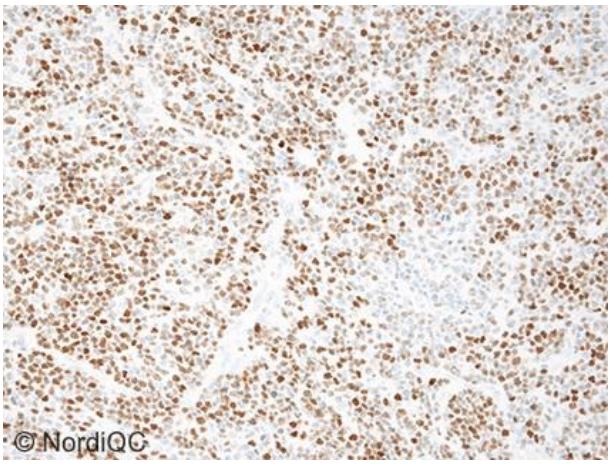


Fig. 3a (x200)
Optimal BCL6 staining reaction of the DLBCL, GCB subtype using same protocol as in Figs. 1a and 2a. All the neoplastic B-cells are distinctively demonstrated showing a moderate to strong nuclear staining reaction.

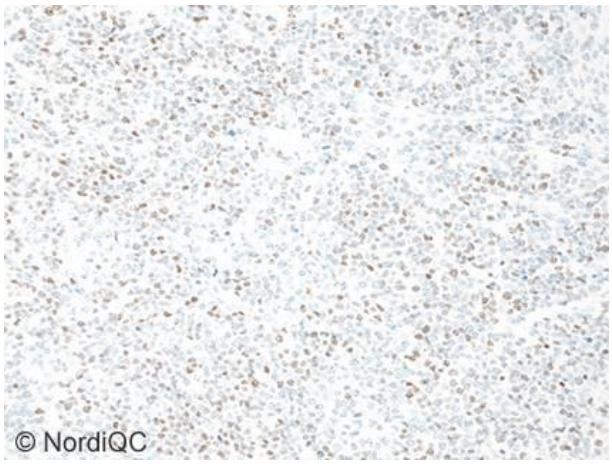


Fig. 3b (x200)
Insufficient BCL6 staining reaction of the DLBCL, GCB subtype using same protocol as in Figs. 1b and 2b. The protocol provided too weak staining intensity and a significant proportion of the neoplastic are false negative - risking misclassification of DLBCL`s in general, using a cut-off value of ≥30% positive neoplastic B-cells according to Hans algorithm[1] (see purpose page 1) – compare with Fig. 3a.

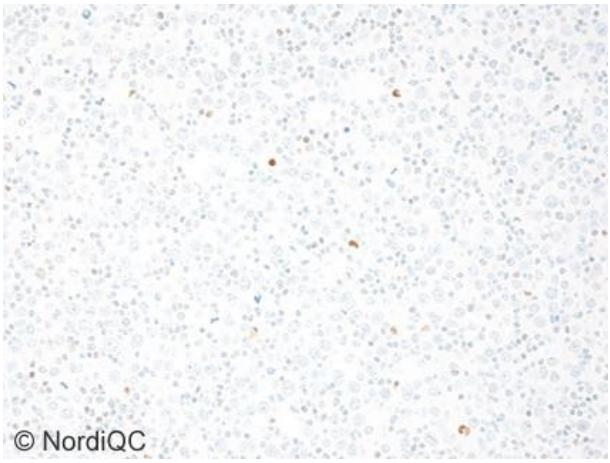


Fig. 4a (x200)
Optimal BCL6 staining reaction of the DLBCL, GCB/ABC subtype using same protocol as in Figs. 1a – 3a. Virtually all neoplastic B-cells are as expected negative. Dispersed normal B-cells intermingling between the neoplastic B-cell are distinctively demonstrated serving as internal positive tissue control.

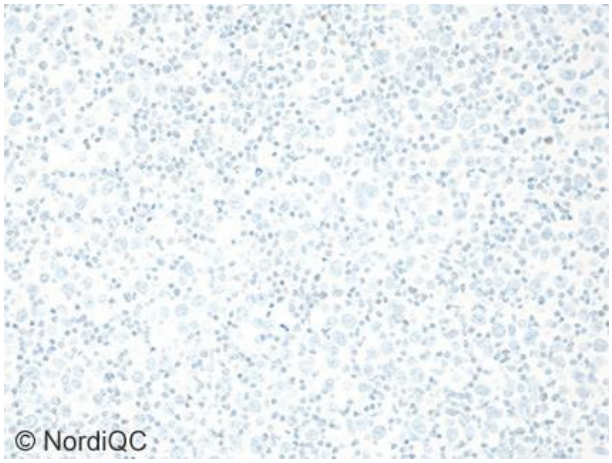


Fig. 4b (x200)
Insufficient BCL6 staining reaction of the DLBCL, GCB/ABC subtype using same protocol as in Figs. 1b – 3b. Although the staining provided the expected reaction pattern, the protocol is challenged giving unreliable results in relation to classification of DLBCL`s, discriminating GCB from non-GCB/ABC subtypes. Also, the dispersed normal B-cells are negative or only sparsely demonstrated – compare with Fig. 4a.

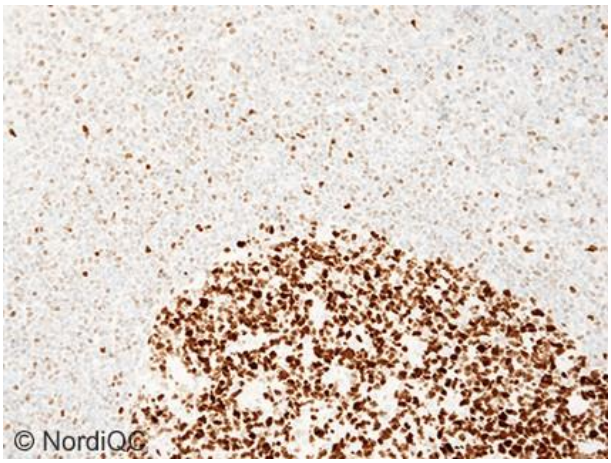


Fig. 5a (x200)
Insufficient BCL6 staining reation of the tonsil (tissue core no. 1) using the RTU product 760-4241 (Ventana/Roche) based the mAb clone GI191/A8, HIER in CC1 (64 min.) and OptiView as detection system. Protocols based on this clone, both concentrated and RTU formats, often gave too much background staining and/or a false positive staining result, compromising interpretation of the specific signals. In this case, the proportion of stained B-cells in the mantle zone of primary follicles is markedly increased - compare with optimal result in Fig. 1a.
Also compare with Fig. 5b, same protocol.

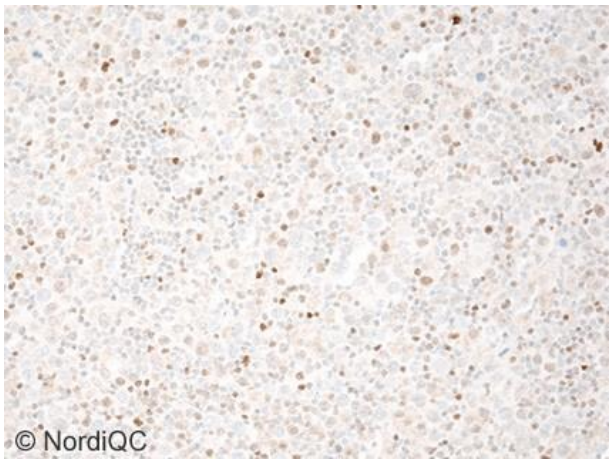


Fig. 5b (x200)
Insufficient BCL6 staining reaction of the DLBCL, non-GCB/ABC subtype using same protocol as in Fig. 5a. The protocol gave excessive background staining including increased proportion of positive neoplastic B-cells expected to be negative, and consequently, risking misclassification not only of DLBCL´s but in principle, all lymphoma subtypes - compare with optimal result in Fig. 4a.

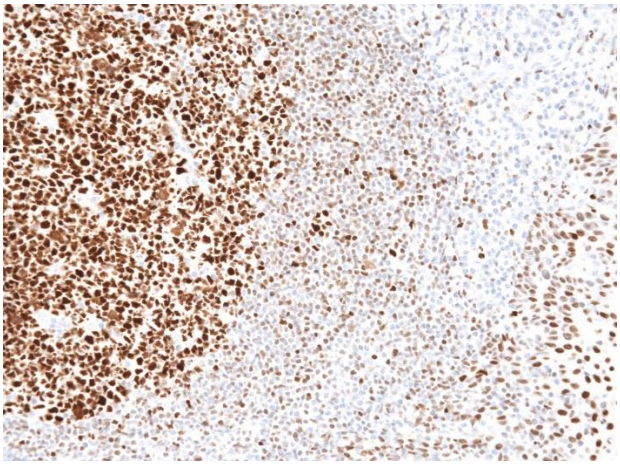


Fig. 6a (x200)
Insufficient BCL6 staining reaction of the tonsil (tissue core no. 1) using the RTU product MAB-0746 (Fuzhou Maixin) based the mAb clone MX042. Virtually all mantle zone B-cells are false positive, hampering interpretation of the specific signal for BCL6 and in general, compromising correct classification of lymphomas e.g., DLBCL`s - see Fig. 6b.

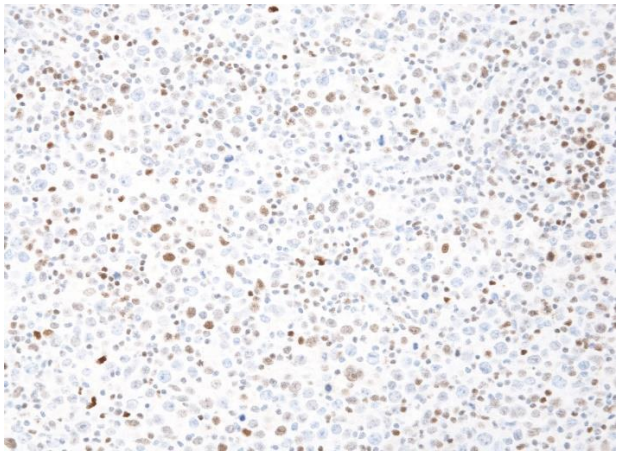


Fig. 6b (x200)
Insufficient BCL6 staining reaction of the DLBCL, non-GCB/ABC subtype using same protocol as in Fig. 6a. The protocol gave a false positive staining reaction of the neoplastic B-cells (weak to strong nuclear staining intensity) and consequently influence interpretation as number of positive neoplastic B-cells are close to the border of the cut-off value (≥30% positive neoplastic B-cells) –compare with optimal result in Fig. 4a.

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