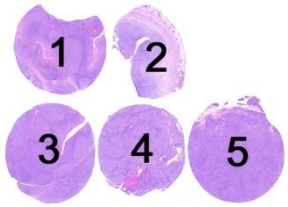
Assessment Run 28 2010

Bcl-2 protein (Bcl-2)

The slide to be stained for Bcl-2 comprised:
1. Tonsil, fixed for 24 h, 2. Appendix, 3. Tonsil, fixed for 48 h, 4. Follicular lymphoma, grade II, 5. Follicular lymphoma, grade III
All tissues were fixed in 10 % neutral buffered formalin.



Criteria for assessing a Bcl-2 staining as optimal included:

- A moderate to strong predominantly cytoplasmic staining of virtually all the peripheral B- and T-cells in the tonsils and appendix.
- An at least weak cytoplasmic staining of the basal squamous epithelial cells of the tonsil and of the basal epithelial cells in the appendix.
- An at least weak to moderate staining of virtually all the neoplastic cells in the two follicular lymphomas.
- No staining reaction in the germinal centre B-cells.

155 laboratories participated in this assessment. 82 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for Bcl-2, run 28**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **124**	98 1	Dako Cell Marque	49	35	15	0	85 %	86 %
mAb clone **100/D5**	5 1 1 1	NeoMarkers Biocare Immunologic Master Diagnostica	2	5	1	0	89 %	100 %
mAb clone **bcl-2/100/D5**	5	Novocastra	3	1	0	1	80 %	-
mAb clone **100**	2	BioGenex	2	0	0	0	-	-
mAb clone **3.1**	2	Novocastra	0	2	0	0	-	-
mAb clone **Bcl-2-100**	1	Zymed	0	0	1	0	-	-
mAb clone **8C8**	1	NeoMarkers	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **124, IR614**	14	Dako	10	4	0	0	100 %	100 %
mAb clone **124, 760-4240**	18	Ventana/Cell Marque	0	8	9	1	44 %	-
mAb clone **124, MON-RTU1011**	1	Monosan	0	0	1	0	-	-
mAb clone **bcl-2/ 100/D5, PA0117**	2	Leica	2	0	0	0	-	-
mAb clone **100/D5, PM003**	1	Biocare	0	1	0	0	-	-
mAb clone **100/D5, 760-2693**	1	Ventana	0	1	0	0	-	-
Total	155		68	58	27	2	-	-
Proportion			44 %	38 %	17 %	1 %	82 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **124**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) in

one of the following buffers: Tris-EDTA/EGTA pH 9 (13/22)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako)(20/28), Target Retrieval Solution pH 6.1 (S1699, Dako)(1/1), Cell Conditioning 1 (BenchMark, Ventana) (4/22), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/4), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1), EDTA/EGTA pH8 (2/3) or Citrate pH 6 (7/14). The mAb was typically diluted in the range of 1:10– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 77 out of 90 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **100/D5**: The protocols giving an optimal result were both based on heat induced epitope retrieval (HIER) in one of the following buffers: EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (1/2). The mAb was typically diluted in the range of 1:50– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings both of 2 laboratories produced an optimal staining.

mAb clone **bcl-2/100/D5**: The protocols giving an optimal result were both based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (3/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:40 depending on the total sensitivity of the protocol employed. Using these protocol settings both of 2 laboratories produced an optimal staining.

mAb clone **100**: The protocols giving an optimal result were both based on heat induced epitope retrieval (HIER) in one of the following buffers: Tris-EDTA/EGTA pH 9 (1/1) or Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1). The mAb was typically diluted in the range of 1:200– 1:1.200 depending on the total sensitivity of the protocol employed. Using these protocol settings both of 2 laboratories produced an optimal staining.

Ready-To-Use Abs
mAb clone **124** (prod. no IR614, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 13 (100 %) laboratories produced a sufficient staining.

mAb clone **bcl-2/100/D5** (prod. no. PA0117, Leica): The protocols giving an optimal result were based on HIER using Bond Epitope Retrieval Solution 1 (Bond, Leica), an incubation time of 25 or 30 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings both of 2 laboratories produced an optimal staining.

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less successful RTU mAb clone 124 (Ventana/Cell Marque, see table 1)
- Too low protocol sensitivity for mAb clone 124 stained on the Ventana BenchMark XT or Ultra platform.

In this assessment and in concordance to the previous assessment of Bcl-2, run 13 2005, the prevalent feature of an insufficient staining was a too weak or false negative staining. This pattern was seen in both the normal peripheral B- and T-cells in the tonsils and the appendix, but also in the neoplastic cells of the two follicular lymphomas and especially in the neoplastic cells of the lymphoma grade III. The mAb clone 124 was the most commonly used marker for Bcl-2. The staining result seemed to be influenced by the automation platform used for the staining. When clone 124 was used on a Ventana BenchMark or Ultra only 21 out of 42 (50%) of the protocols gave a sufficient staining, and only 4 protocols resulted in an optimal staining. The protocols giving an optimal result were typically based on a high concentration of the clone (1:10 – 1:20), efficient HIER by Standard CC1, and UltraView + amplification as the detection system. No optimal results were obtained when the clone was applied as a RTU format (Ventana/Cell Marque). When the same clone was used on, e.g., the Dako Autostainer platform 59 out of 61 (97%) of the protocols gave a sufficient result. The mAb could both be used as a concentrate, typically diluted in the range of 1:50 – 400, and in an RTU format.

Tonsil appeared to be a suitable control: In the optimally calibrated protocols the majority of the basal squamous epithelial showed a weak to moderate but distinct cytoplasmic staining, indicating that these cells may serve as a reliable positive critical stain quality indicator (CSQI) for Bcl-2.

This was the 2nd assessment of Bcl-2 in NordiQC, as Bcl-2 also was assessed in run 13, 2005 (Table 2). The lower pass rate in the current run is probably due to more challenging tissue material circulated, a significant increase in the number of new participants, and a larger number of laboratories using clone 124 with a Ventana system.

Table 2. **Proportion of sufficient results for Bcl-2 in the two NordiQC runs performed**

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

Conclusion

The mAbs clones 124, 100, 100/D5, bcl-2/100/D5 and 3.1 can all be used to obtain an optimal staining of Bcl-2. For all 5 mAbs HIER is mandatory to obtain an optimal staining. The concentration of the mAb clone 124 is highly dependant of the stainer applied. Tonsil is an appropriate control: An at least weak but distinct cytoplasmic reaction must be seen in the majority of the basal squamous epithelial cells, while the germinal centre B-cells should be negative.

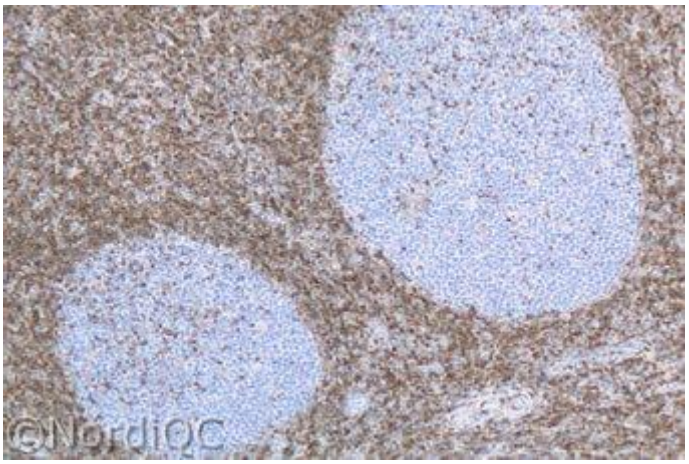


Fig. 1a
Optimal Bcl-2 staining of the tonsil using the mAb clone 124 optimally calibrated after HIER in an alkaline buffer. Virtually all the peripheral B- and T-cells show a strong staining. In the germinal centres, scattered T-cells show a distinct staining, whereas the B-cells are negative – also compare with Figs. 2a & 3a, same protocol.

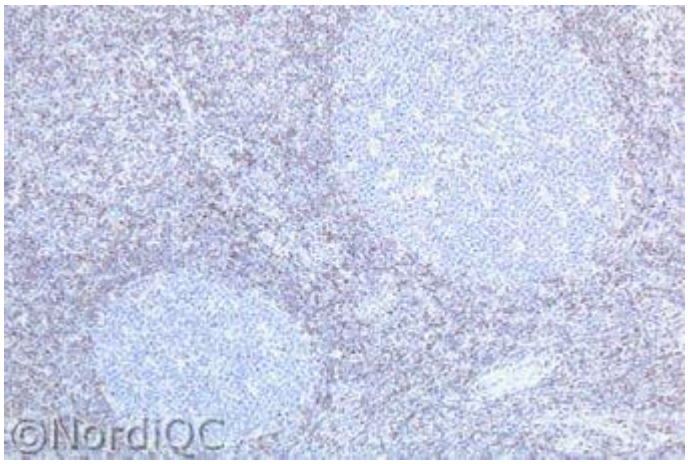


Fig. 1b
Insufficient Bcl-2 staining of the tonsil using the mAb clone 124 too diluted - same field as in Fig. 1a. The intensity and proportion of cells is significantly reduced compared to the expected level – also compare with Figs. 2b & 3b, same protocol.

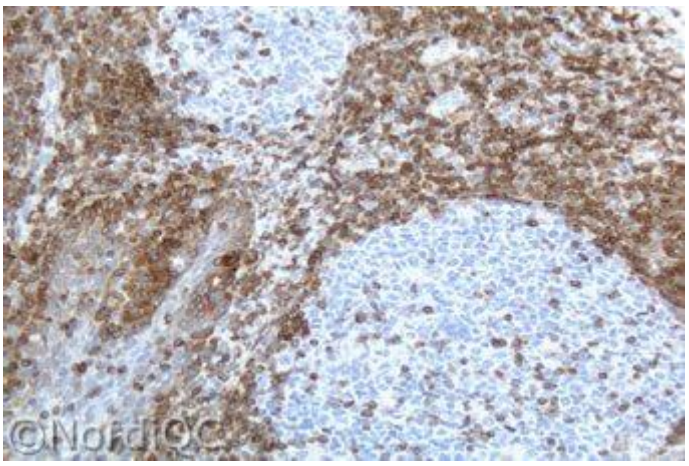


Fig. 2a
High magnification of the optimal Bcl-2 staining of the tonsil shown in Fig. 1a. The scattered T-cells within the germinal centre show a distinct staining and also the basal squamous epithelial cells (left) show a weak to moderate staining. Same protocol as in Fig. 1a.

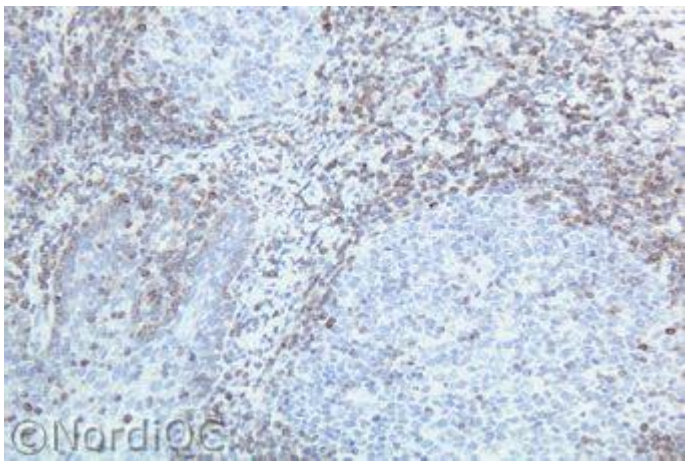


Fig. 2b
High magnification of the insufficient Bcl-2 staining of the tonsil shown in Fig. 1b – same field as in Fig. 2a.
Only the grouped peripheral lymphocytes show a distinct staining, while the germinal centre T-cells and the basal squamous epithelial cells virtually are negative. Same protocol as in Fig. 1b.

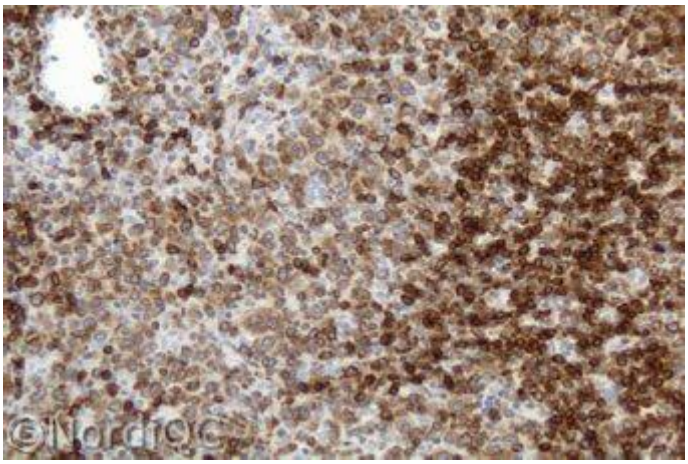


Fig. 3a
Optimal Bcl-2 staining of the follicular lymphoma grade III using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic show a moderate staining, while the remnants of the normal lymphocytes (right) show a strong staining.

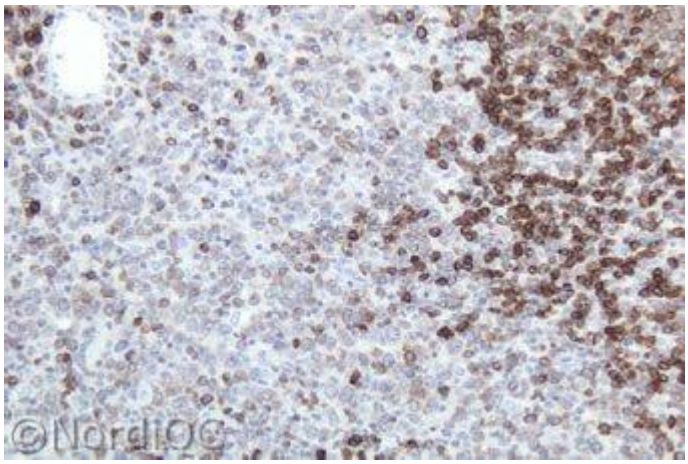


Fig. 3b
Insufficient Bcl-2 staining of the follicular lymphoma grade III using same protocol as in Figs. 1b & 2b. – same field as in Fig. 3a. The normal lymphocytes show a moderate staining, while the neoplastic cells only show a weak, equivocal staining.

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