Assessment Run 17 2006

Bcl-6

The slide to be stained for bcl-6 comprised:
1. Tonsil fixed 24 h, 2. Tonsil fixed 72 h, 3. Follicular lymphoma, 4 - 5. Diffuse large B-cell lymphomas, 6. Follicular lymphoma. All specimens were fixed in 10 % NBF.

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

- A moderate to strong distinct nuclear staining of the majority of the normal germinal centre cells and the squamous epithelial cells in the two tonsils.
- A moderate to strong distinct nuclear staining of the two follicular lymphomas.
- A strong nuclear staining of the no. 5 diffuse large B-cell lymphoma (Germinal Centre B-Cell Like - GCL)
- Negative staining of the no. 4 diffuse large B-cell lymphoma (Activated B-Cell like - ABC).

69 laboratories submitted stains. At the assessment 29 achieved optimal marks (42 %), 31 good (45 %), 6 borderline (9 %) and 3 (4 %) poor marks.

The following Abs were used:
mAb clone **PG-B6p** (Dako, n=62)
mAb clone **GI 191E/A8** (Ventana, n=2; Cell Marque, n=1)
mAb clone **P1F6+PG-B6p** (NeoMarkers, n=2)
mAb clone **BL6.02** (NeoMarkers, n=1)
mAb clone **P1F6** (Novocastra, n=1)

Optimal staining for bcl-6 in this assessment was obtained with the mAb clone **PG-B6p** (29 out of 62) and the mAb clone **GI 191E/A8** (1 out of 3). All optimal protocols were based on Heat Induced Epitope Retrieval (HIER). No difference in staining intensity between the two tonsils fixed 24 h and 72 h, respectively, was seen.

With clone **PG-B6p** all protocols giving an optimal staining were based on HIER using an alkaline buffer (Tris-EDTA/EGTA pH 9, Cell Conditioning 1 [CC1, Ventana] or EDTA pH 8). Clone PG-B6p was typically used in the range of 1:10 – 1:40 with a 2-step polymer based detection system such as EnVision+ (Dako) or a 3-step biotin based system such as IView (Ventana). With a more sensitive 3-step polymer system such as Powervision+, Immunovision or Advance (Dako), PG-B6p was typically diluted in the range of 1:25 - 1:100. The choice of detection system did not appear to influence the proportion of optimal results.

With clone **GI 191E/A8** as a RTU Ab visualized with Multimer, Ventana, the protocol resulting in an optimal result was based on HIER using CC1.

The most frequent causes of insufficient staining were:
- Insufficient HIER using citrate pH 6 as the heating buffer
- Too low concentration of the primary antibody
- Less successful primary antibody

In the assessment the prevalent feature of an insufficient staining was a too weak or false negative staining of the neoplastic B-cells in the two follicular lymphoma and the normal germinal centre cells in the tonsils. The majority of laboratories was capable to detect bcl-6 in the GCL diffuse large B-cell lymphoma.

Normal tonsil is a reliable control for the demonstration of bcl-6. The majority of the germinal centre cells should show a strong nuclear staining with no cytoplasmic reaction. The mantle zone B-cells should be negative.

Conclusion
The mAb clones **PG-B6p** and **GI 191E/A8** are useful antibodies for bcl-6. HIER in an alkaline buffer (e.g., Tris-EDTA/EGTA pH 9 or CC1) is mandatory for optimal performance. Normal tonsil fixed in 10 % NBF for of 24 – 72 hours is a suitable control.

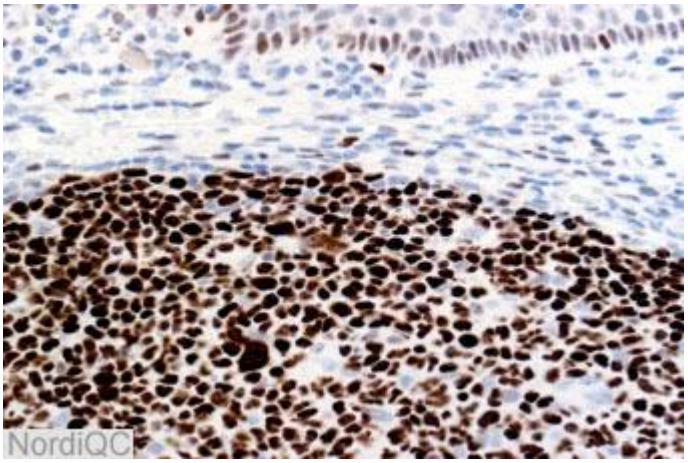

Fig. 1a
Optimal staining for bcl-6 of the tonsil. The germinal centre B-cells and the basal squamous epithelial cells show a distinct nuclear staining and no background staining.

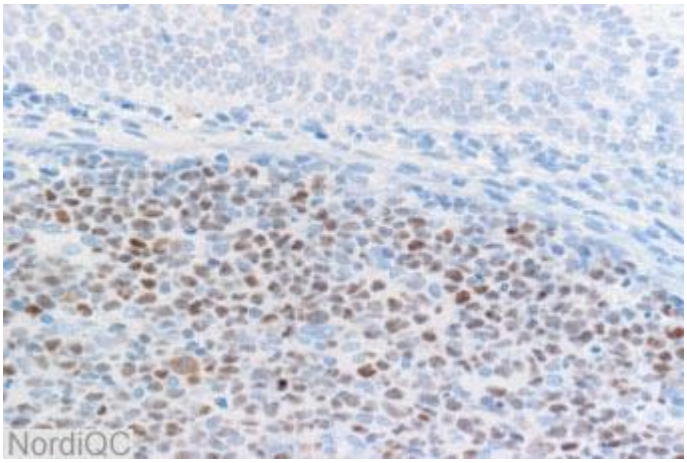

Fig. 1b
Staining for bcl-6 of the tonsil using an insufficient protocol (same field as in Fig. 1a.). The majority of the germinal centre cells expected to cells are demonstrated, however compare with Fig. 2b – same protocol.

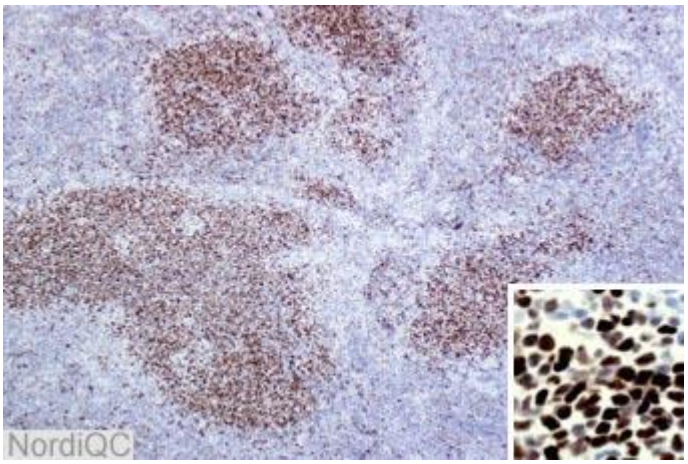

Fig. 2a
Optimal staining for bcl-6 of the follicular lymphoma. Virtually all the neoplastic cells show a strong and distinct staining (same protocol used in Fig. 1a).

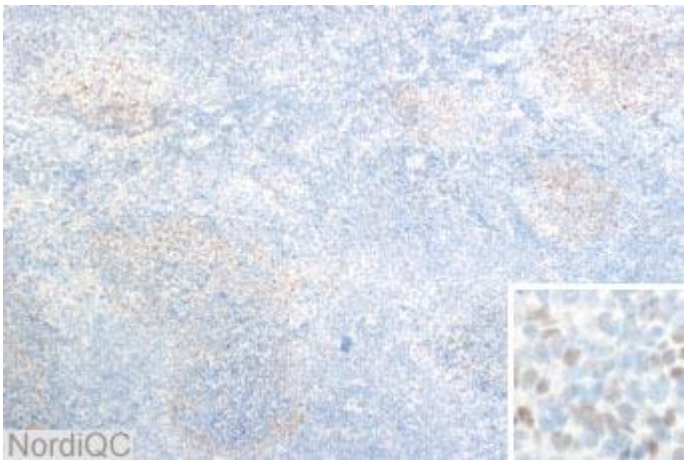

Fig. 2b
Insufficient staining for bcl-6 of the follicular lymphoma (same field as in Fig 2a). All the neoplastic cells are only weakly positive or totally negative (same protocol used in Fig. 1b).

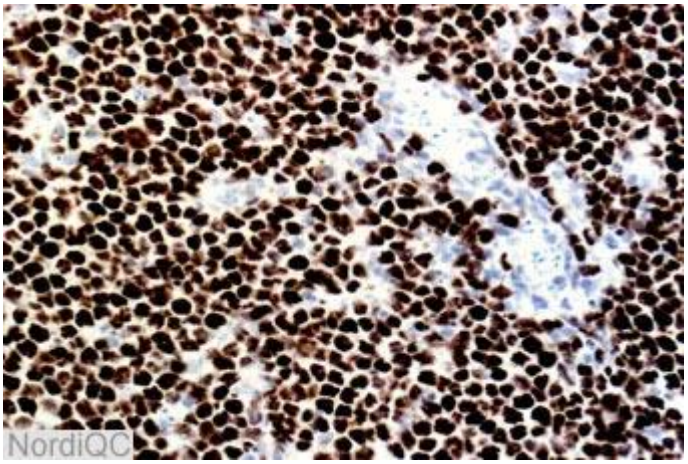

Fig. 3a
Optimal staining for bcl-6 of the germinal cell like diffuse large B-cell lymphoma. Virtually all the neoplastic cells show a strong and distinct nuclear staining (same protocol used in Fig. 1a).

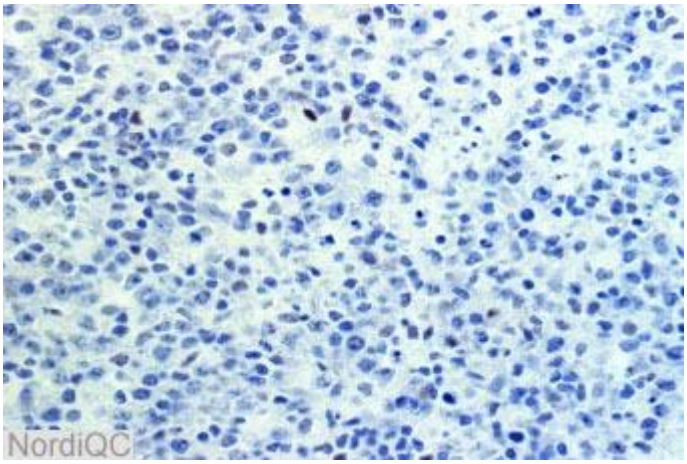

Fig. 3b
Optimal staining for bcl-6 of the activated B-cell like diffuse large B-cell lymphoma. Virtually all the neoplastic cells are negative and only a few isolated cells are stained (same protocol used in Fig. 1a).

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