Assessment Run 13 2005

Bcl-2 protein

The slide to be stained for Bcl-2 comprised:
1: Tonsil fixed for 24 h., 2: Tonsil fixed for 168 h., 3 - 5: Follicular lymphoma. All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Bcl-2 staining as optimal included: In both tonsils a moderate to strong distinct cytoplasmic staining of the peripheral B-lymphocytes in the mantle-zone and T-lymphocytes in the interfollicular areas and germinal centres (whereas the germinal B-lymphocytes should be negative) and a strong staining of all the follicular lymphomas.

87 laboratories participated in the assessment, of which 53 achieved optimal (61%), 28 good (32%), 4 borderline (5%) and 2 poor marks (2%).

The following antibody clones were used:
mAb clone **124** (DakoCytomation, n=70)
mAb clone **Bcl2/100/D5** (Ventana, n=5; NovoCastra, n=4; Medac, n=1)
mAb clone **100** (BioGenex, n=4)
mAb clone **Bcl-2-100** (Zymed, n=2)
mAb clone **8C8** (NeoMarkers, n=1).

Optimal stains in this assessment were obtained with the clones 124 (43/70), Bcl2/100/D5 (6/10), 100 (3/4) and 8C8 (1/1).

All laboratories achieving an optimal result used HIER. All of the heating buffers Tris-EDTA/EGTA pH 9 (43/63), Citrate pH 6 – 7,2 (5/9), CC1 Ventana Benchmark (1/7), EDTA pH 8 (2/5), TRS low pH 6.1 S1699 DakoCytomation (1/2) and TRS high pH 9.9 S3307 DakoCytomation (1/1) could be used to obtain an optimal staining.

The mAb clone 124 was typically used in the range of 1:25 - 800 and resulted in an optimal staining using one of the above mentioned HIER buffers. MAb clone Bcl2/100/D5 was used in the range of 1:20 – 100 with Tris-EDTA/EGTA pH 9 or Citrate pH 6. MAb clone 100 was used in the range of 1:300 – 500 with Tris-EDTA/EGTA pH 9 or Citrate pH 6, and mAb clone 8C8 was used in 1:500 with Citrate pH 6.

The most frequent cause of insufficient staining was:
- Too diluted primary antibody.

The prevalent feature of an insufficient staining was a too weak or completely negative reaction in the tonsils and malignant lymphomas. Typically there was no difference in the intensity in the normal and neoplastic lymphocytes indicating that the protein expression in these conditions is comparable, which enables the laboratories to use normal tonsil as a reliable control for Bcl-2 in the classification of lymphoma. In order to verify the sensitivity and specificity the germinal centres are optimal. The isolated T-lymphocytes in the germinal centres should be clearly demonstrated while the germinal B-lymphocytes should be completely negative.

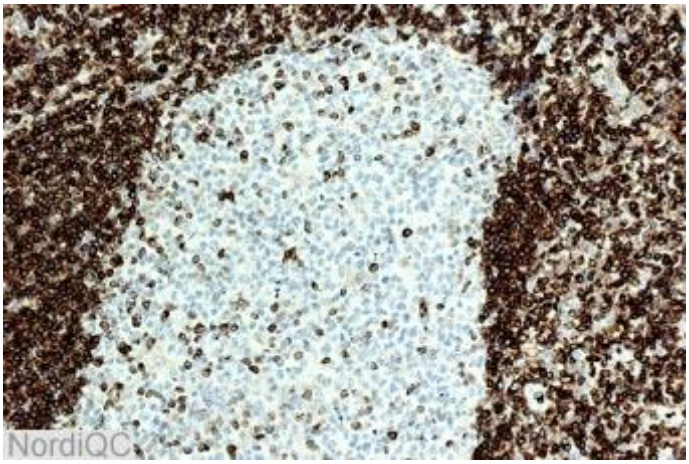

Fig. 1a
Optimal staining for Bcl-2 in a normal tonsil. The mantle zone lymphocytes show a strong cytoplasmic staining. The germinal centre cells are negative except the isolated T-lymphocytes.

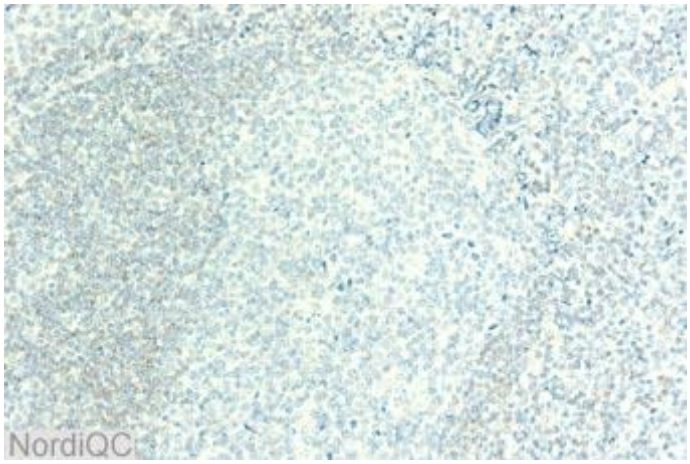

Fig. 1b
Insufficient staining for Bcl-2 in a normal tonsil (same field as in Fig. 1a). The mantle zone lymphocytes are only weakly stained.

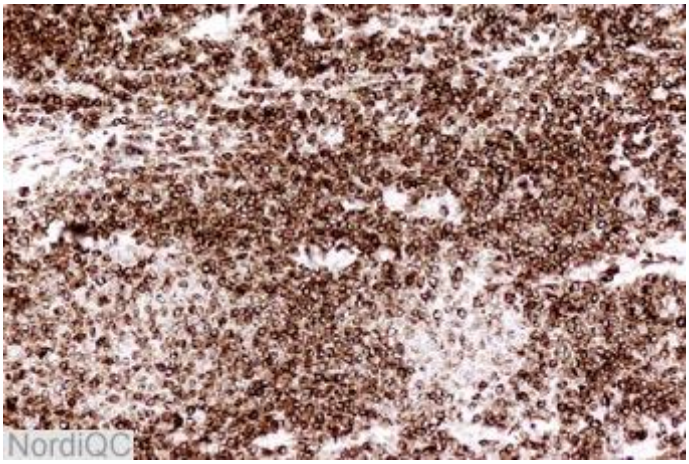

Fig. 2a
Optimal staining for Bcl-2 in a follicular lymphoma. Almost all the neoplastic cells show a strong and distinct staining.

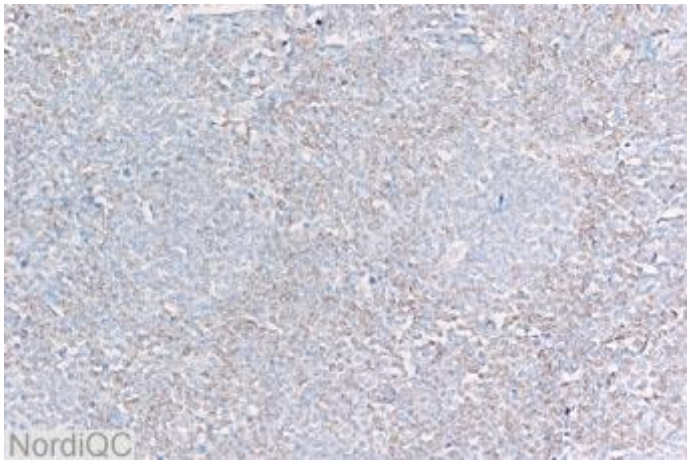

Fig. 2b
Insufficient staining for Bcl-2 in a follicular lymphoma (same field as in Fig. 2a). The neoplastic cells are only weakly stained.

SN/MV/LE 4-4-2005