Assessment Run 14 2005

CD8

The slide to be stained for CD8 comprised:
1: Liver, 2: Tonsil fixed 4 h, 3: Tonsil fixed 48 h, 4: Tonsil fixed 96 h, 5-7: Nodal T-cell lymphomas.

Criteria for assessing a CD8 staining as optimal included:

- A strong and distinct predominantly membranous as well as cytoplasmic granular reaction of the neoplastic cells in one of the T-cell lymphomas (no. 6), whereas the neoplastic cells in the two other T-cell lymphomas should be negative.
- A strong and distinct membranous as well as cytoplasmic granular staining of the normal suppressor/cytotoxic T-cells in all of the tonsils and the liver.
- No staining in other cells. Especially B-cells in the tonsil should be negative

63 laboratories participated in the assessment. 41 achieved optimal staining (66 %), 17 good (26 %), 4 borderline (6 %) and 1 (2 %) poor staining.

The following Abs were used:
mAb clone **C8/144B** (DakoCytomation n=41, NeoMarkers n=2)
mAb clone **4B11** (Novocastra n=15)
mAb clone **1A5** (Ventana n=3, Novocastra n=1)
mAb clone **DK25** (DakoCytomation n=1)

Optimal staining in this assessment was obtained with following Abs: clone **C8/144B** (30 out of 42 were optimal), clone **4B11** (7 out of 15 were optimal) and clone **1A5** (4 out of 4 were optimal).

All optimal protocols were based on HIER irrespectively of the Ab employed.

With clone **C8/144B** all of the following could be used as the heating buffer: Tris-EDTA/EGTA pH 9 (25 out of 30 were optimal), EDTA/EGTA pH 8 (1 out of 2 was optimal), CC1,Ventana (2 out of 4 were optimal), TRS pH 9.9, DakoCytomation (1 out of 1 was optimal) and Citrate pH 6 (1 out of 4 were optimal). **C8/144B** was typically diluted in the range of 1:50 – 1:200.
With clone **4B11** only Tris-EDTA/EGTA pH 9 could be used (7 out of 12 were optimal). 4B11 was typically diluted in the range of 1:25 – 1:100.
With clone **1A5** all of the following could be used as the heating buffer: CC1, Ventana (2 out of 2 were optimal), Tris-EDTA pH 9 (1 out of 1 was optimal) and EDTA/EGTA pH 8 (1 out of 1 was optimal). 1A5 could both be used as a Ready-To-Use product (3 out of 3) or as a concentrated Ab diluted 1:80.

The most frequent causes of insufficient staining were (often in combination):
- Too low concentration of the primary antibody
- Insufficient HIER

The prevalent feature of an insufficient staining was a too weak and diffuse staining of the normal suppressor/cytotoxic T-cells as well as neoplastic T-cells. There was no significant difference in the staining characteristics of the normal and the neoplastic T-cells, indicating that tonsil can be used as reliable control, in which both the isolated and grouped T-cells should be distinctively demonstrated.

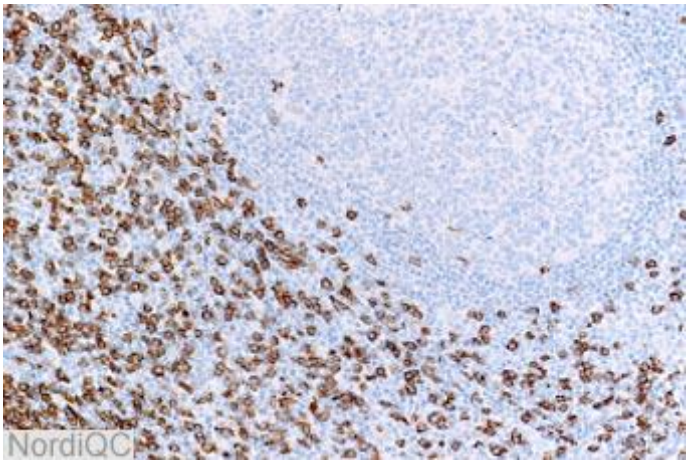

Fig. 1a
Optimal staining for CD8 in a normal tonsil. The interfollicular T-cells are distinctively demonstrated.

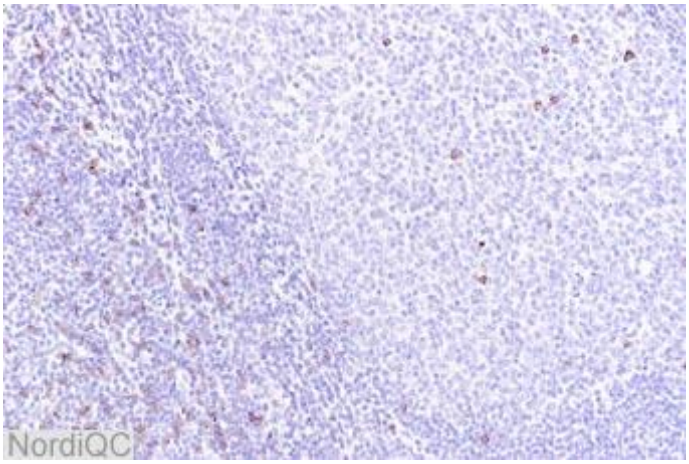

Fig. 1b
Insufficient staining for CD8 in a normal tonsil (same field as in Fig. 1a). The T-cells are either unstained or only weakly stained (compare to Fig. 1a).

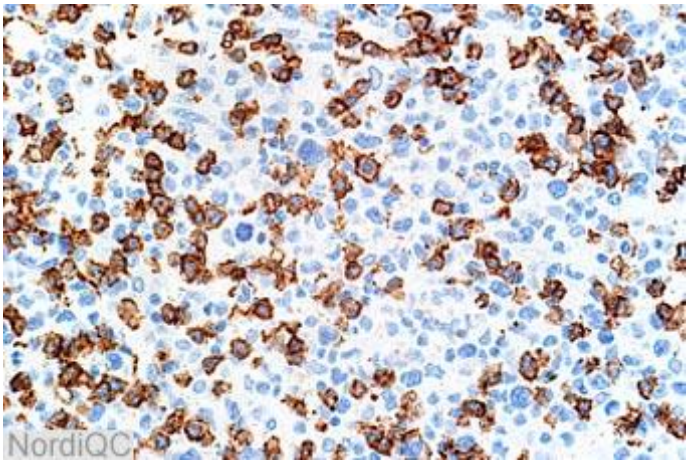

Fig. 2a
Optimal staining for CD8 in a T-cell lymphoma. Both residual normal T-cells and many neoplastic T-cells are demonstrated. The reaction is both membranous and cytoplasmic granular.

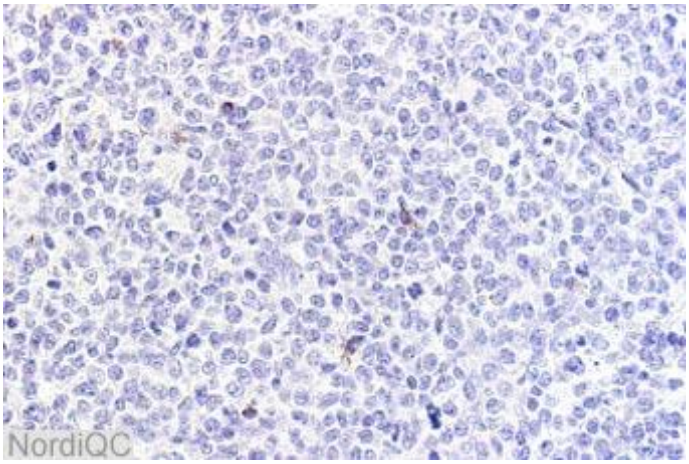

Fig. 2b
Insufficient staining for CD8 in a T-cell lymphoma (same field as in Fig. 2a). The neoplastic cells are virtually negative and only a few normal T-cells are weakly stained.

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