Assessment Run 14 2005

CD4

The slide to be stained for CD4 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 CD4 staining as optimal included:

- A strong, distinct, predominantly membranous reaction of the neoplastic cells in the T-cell lymphomas no. 5 and 7. The T-cell lymphoma no. 6 was CD4 negative and only remnants of normal T-cells should be demonstrated.
- A strong, distinct, predominantly membranous staining of the normal helper/inducer T-cells in all of the tonsils.
- A moderate, distinct staining of macrophages, in particular the germinal centre macrophages in the tonsils, Kupffer cells in the liver and the endothelial cells of the liver sinusoids.
- No staining in other cells. Especially the B-cells should be negative.
- A nuclear reaction observed with the clone 1F6 was accepted, as this did not interfere with the interpretation.

59 laboratories participated in the assessment. 18 achieved optimal staining (31 %), 24 good (41 %), 8 borderline (14 %) and 9 (15 %) poor staining.

The following Abs were used:
mAb clone **1F6** (BioCare n=1, NeoMarkers n=4, Novocastra n=30, Ventana n=5)
mAb clone **4B12** (NeoMarkers n= 5, Novocastra n=14)

Optimal staining in this assessment could be obtained with both clone **1F6** (9 out of 40) and clone **4B12** (9 out of 19).

All optimal protocols with the two Abs were based on HIER. For obtaining an optimal result with clone **1F6** the following heating buffers could be used: Tris-EDTA/EGTA pH 9 (6 out of 24 were optimal), EDTA/EGTA pH 8 (2 out of 5 were optimal) and CC1, Ventana (1 out of 7 were optimal).

1F6 was typically either diluted in the range of 1:10 – 1:25 (in which 7 out of 27 were optimal) or used as a Ready-To-use Ab (2 out of 6 were optimal).

The relative low rate of optimal stains might indicate, that other methodological parameters could affect the staining result. As regards clone **1F6**, according to vendor recommendations, blocking of endogenous peroxidase in >1 % H_2O_2 after HIER can be deteriorative to the antigen detected. This has been confirmed in several NordiQC reference laboratories (see Figs. 3a-3b). Thus it is recommended either to use a lower concentration of H_2O_2, performing the peroxidase blocking before HIER or omit endogenous peroxidase blocking at all. 3 out of 3 laboratories omitting peroxidase blocking or performing the step before HIER obtained good marks.

For obtaining an optimal result with clone **4B12** the following heating buffers could be used: Tris-EDTA/EGTA pH 9 (6 out of 14 were optimal), EDTA/EGTA pH 8 (2 out of 3 were optimal) and TRS 9,9, DakoCytomation (1 out of 1 was optimal). **4B12** was typically diluted in the range of 1:25 – 1:400 (in which 9 out of 17 were optimal).

The most frequent causes of insufficient staining were (often in combination):
- Too low concentration of the primary antibody
- Insufficient HIER (i.e. using Citrate pH 6 as HIER buffer or a too short HIER time, i.e., < 15 efficient heating time in a MWO)
- Possibly inappropriate blocking of endogenous peroxidase with the clone **1F6** (10 laboratories using the same protocol as used for an optimal staining, obtained an insufficient result, however, we have not yet obtained data regarding this question)

The prevalent feature of an insufficient staining was a too weak and diffuse staining or almost completely

negative reaction of both the neoplastic T-cells and the normal helper T-cells. A good quality indicator in this assessment was the germinal centre macrophages, as these cells typically were demonstrated in a staining assessed as optimal or good, while the cells were too weak or negative in staining assessed as borderline or poor.

Compared to normal helper/inducer T-cells the macrophages generally express a weak CD4 staining. The capability to detect these cells verifies the sensitivity of the used protocol.

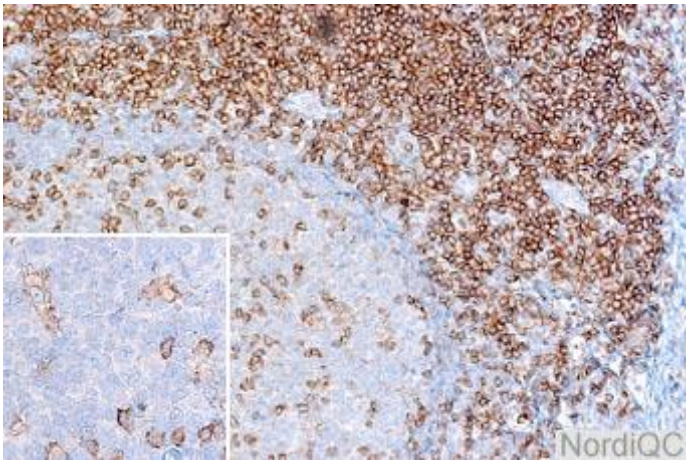


Fig. 1a
Optimal staining for CD4 in a normal tonsil. The majority of the T-cells show a strong membranous staining. Both grouped and isolated T-cells and - more importantly - germinal macrophages (insert) are demonstrated.

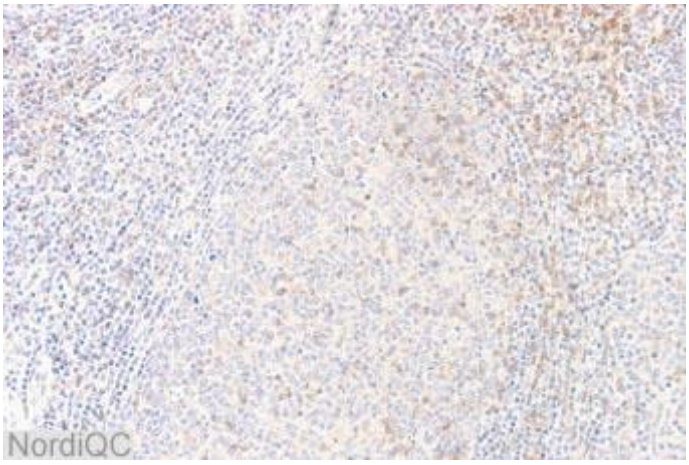


Fig. 1b
Insufficient staining for CD4 in a normal tonsil. The T-cells only show a diffuse and weak staining. Both isolated T-cells and germinal macrophages are negative.

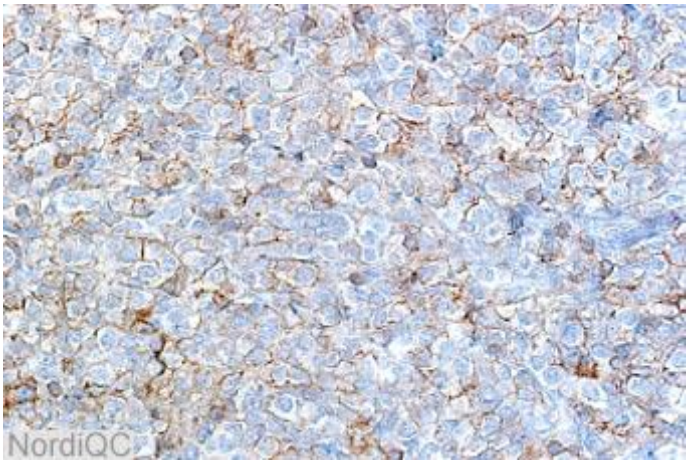


Fig. 2a
Optimal staining for CD4 in a T-cell lymphoma. The majority of the neoplastic cells show a moderate to strong and distinct membranous staining.

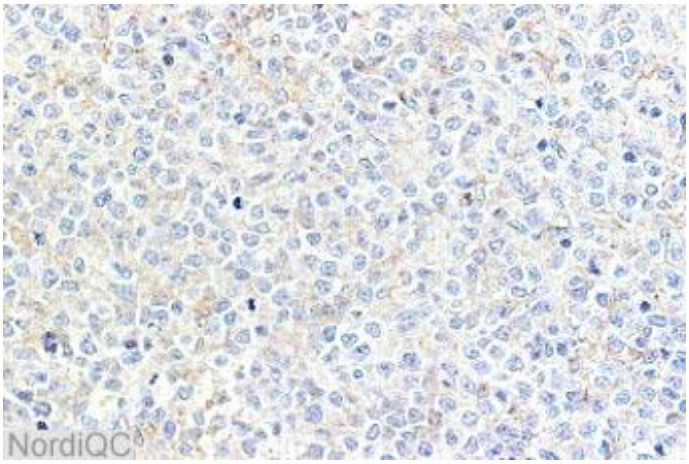
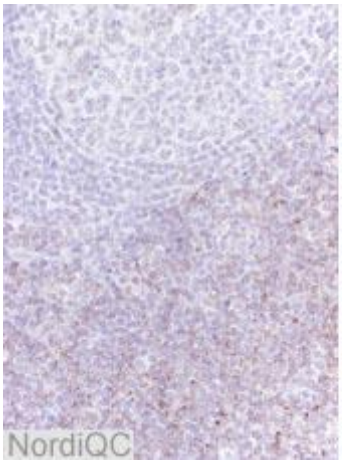


Fig. 2b
Insufficient staining for CD4 in a T-cell lymphoma (same field as in Fig. 2a). The neoplastic cells are only weakly stained.



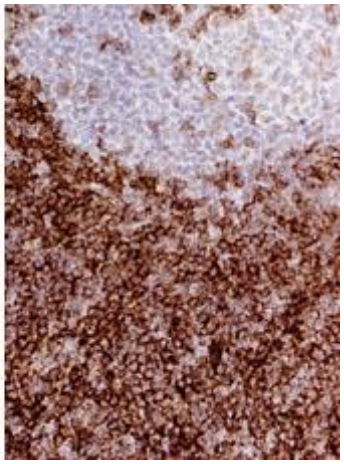
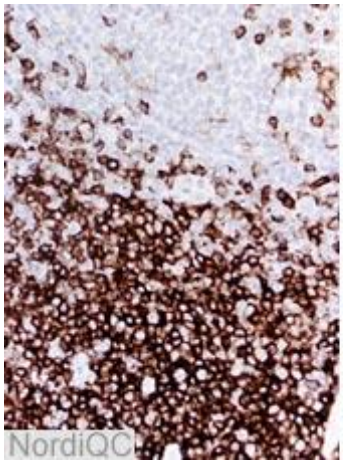


Fig. 3a
Left: Staining for CD4 in a tonsil using the clone **1F6** with endogenous peroxidase blocking in 3 % H2O2 after HIER and before incubation in primary Ab. The T-cells only show a diffuse and weak staining.
Right: Staining for CD4 in a tonsil using the clone 1F6 with endogenous peroxidase blocking in 3 % H2O2 before HIER and before incubation in primary Ab. The T-cells are strongly labelled.



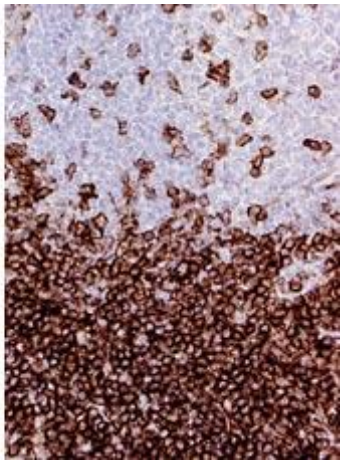


Fig. 3b
Left: Staining for CD4 in a tonsil using the clone **4B12** with endogenous peroxidase blocking in 3 % H2O2 after HIER and before incubation in primary Ab.
Right: Staining for CD4 in a tonsil using the clone 4B12 with endogenous peroxidase blocking in 3 % H2O2 before HIER and before incubation in primary Ab.
In both protocols the T-cells are strongly labelled.

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