Assessment Run 15 2005

CD45 (Leucocyte Common Antigen)

The slide to be stained for **CD45** (Leucocyte Common Antigen) comprised:
1. Tonsil (fixed 48 h), 2. Liver, 3. Brain, 4. Lymph node with chronic lymphatic leukaemia (CLL), 5. Malignant melanoma.
All specimens were fixed in 10 % NBF.

Criteria for assessing a CD45 staining as optimal included:

- A strong and distinct predominantly membranous staining of the normal lymphocytes (both B- and T-cells) in all of the specimens.
- At least a weak but distinct staining of Kupffercells in the liver and microglia in the brain.
- A strong and distinct predominantly membranous staining of the majority of the neoplastic cells in the CLL.
- No staining of the malignant melanoma.

86 laboratories submitted stains. 6 laboratories used an antibody considered inappropriate (i.e., not detecting CD45). Assessing the remaining, 34 achieved optimal marks (43 %), 34 good (43 %), 11 borderline (13 %) and 1 (1 %) poor marks.

The following Abs were used:
mAb clone **2B11+PD7/26** (Dako, n=65)
mAb clone **RP2/18** (Ventana, n=8)
mAb clone **PD7/26/16+2B11** (NeoMarkers, n=3)
mAb clone **PD7/26** (Dako, n=2)
mAb clone **X16/99** (Novocastra, n=1)
mAb clone **T29/33** (Dako, n=1)

The following Abs were considered inappropriate, as they only demonstrate subtypes of either B- or T-cells and thus is not a marker for leucocyte common antigen: mAb clone **4KB5** (which detects CD45RA primarily present in the majority of B-cells) and mAb clone **UCHL1** (which detects CD45R0 primarily present in the majority of T-cells).

Optimal staining for CD45 in this assessment was obtained with following mAbs: clone: **2B11+PD7/26** (30 out of 65), **RP2/18** (3 out of 8) and **T29/33** (1 out of 1).

The optimal protocols were based on Heat Induced Epitope Retrieval (HIER) (33 out of 34) and no pre-treatment (1 out of 1).

With clone **2B11+PD7/26** the following HIER buffers were used in the optimal protocols: Tris-EDTA/EGTA pH 9 (22 out of 46 were optimal), CC1 (Ventana) (4 out of 4 were optimal), Citrate pH 6 (2 out of 12 were optimal) and Target Retrieval Solution S1699 (Dako, 1 out of 1 was optimal). Omission of pre-treatment was used in one optimal protocol (1 out of 3 was optimal). In the optimal protocols clone **2B11+PD7/26** was typically used in the range of 1:100–1:500 depending on the sensitivity of the protocol applied.

With clone **RP2/18** only CC1 (Ventana) could be used as HIER buffer in the optimal protocols (3 out of 6 were optimal). In all optimal protocols **RP2/18** was applied as a ready-to-use Ab.

With clone **T29/33** citrate pH 6 was used as HIER buffer in the optimal protocol. **T29/33** was diluted 1:800.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Inappropriate epitope retrieval (proteolysis)
- Omission of epitope retrieval
- Less successful primary Abs

In the assessment the prevalent feature of an insufficient staining was a too weak staining of both the normal lymphocytes and the CLL. In the insufficient staining the membranous staining of the lymphocytes was indistinct and diffuse.
A good quality indicator was the ability to demonstrate CD45 in macrophages including Kupffer cells and microglial cells. In all protocols giving an optimal and good staining these macrophages were demonstrated whereas they were largely negative in the insufficient staining.

Conclusion
The mAb clones **2B11+PD7/26** and **RP2/18** seems to be the most appropriate markers for CD45.
HIER is highly recommended for optimal performance of both clones.
As a supplement to tonsil, in which all lymphocytes should be demonstrated, liver seems to a good control for CD45: The Kuppfer cells, which have a relative low CD45 expression, should be demonstrated.

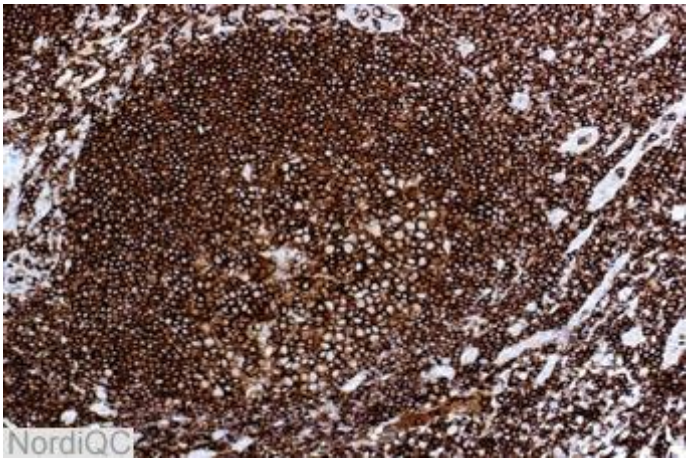

Fig. 1a
Optimal staining for CD45 in the tonsil. All lymphocytes (B-and T-cells) show a strong membranous reaction.

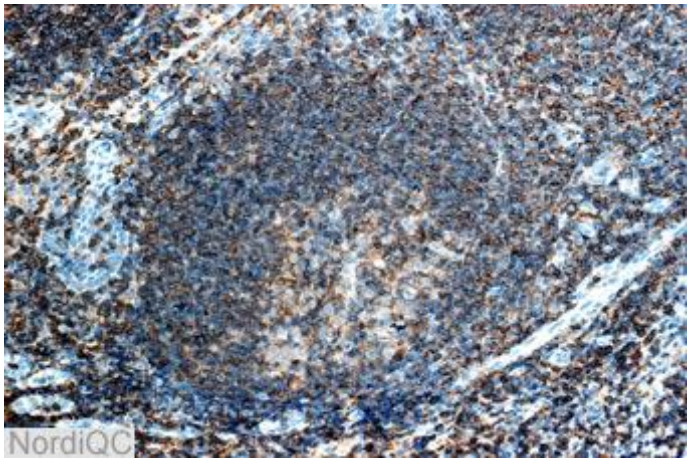

Fig. 1b
Staining for CD45 in the tonsil using an insufficient protocol (same field as in Fig. 1a.). The majority of lymphocytes are demonstrated. However, compare with Fig. 2b and Fig. 3b – same protocol.

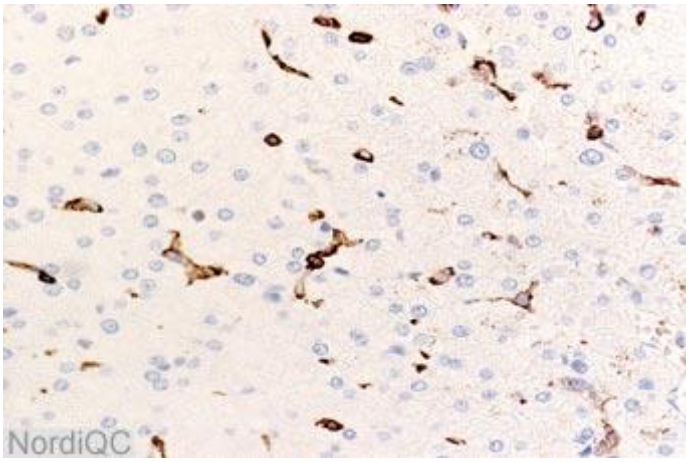

Fig. 2a
Optimal staining for CD45 in the liver. The lymphocytes and – more important – the Kupffer cells show a distinct membranous reaction.

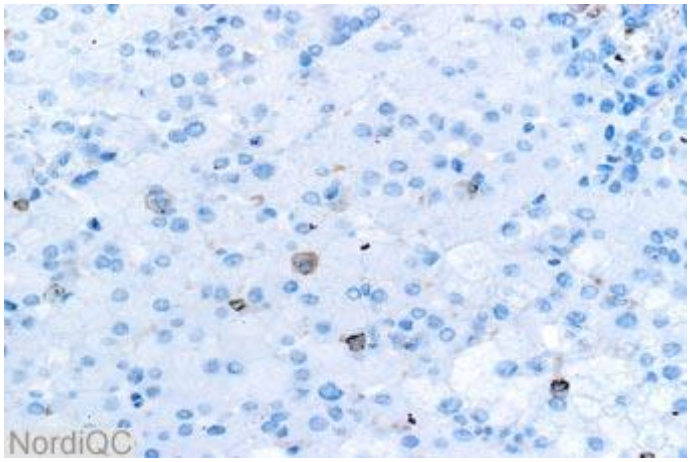

Fig. 2b
Insufficient staining for CD45 in the liver. Only the lymphocytes are demonstrated while the kupffer cells are negative (same protocol as used in Fig. 1b).

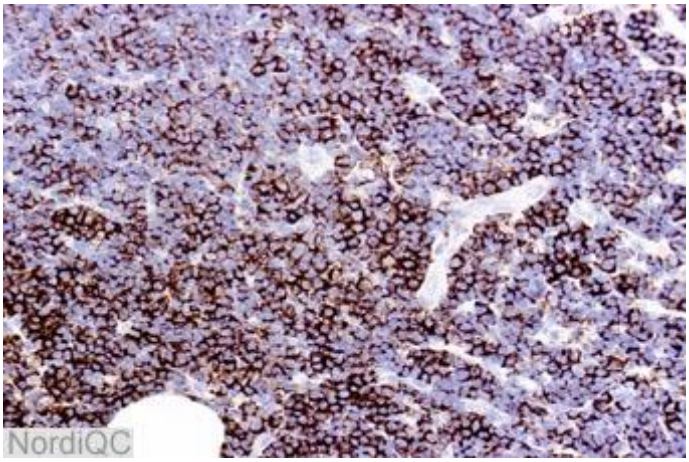

Fig. 3a
Optimal staining for CD45 in the CLL. The majority of the neoplastic cells show a strong distinct membranous reaction (same protocol as used in Fig. 1a and 2a).

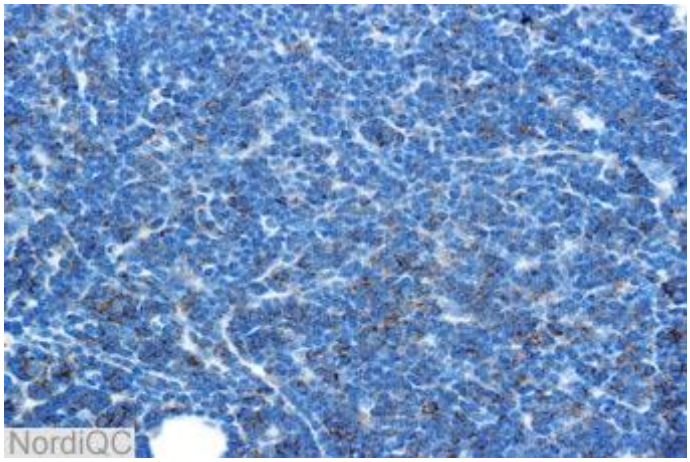

Fig. 3b
Insufficient staining for CD45 in the CLL. The majority of the neoplastic cells are unstained or only weakly positive (same protocol as used in Fig. 1b and 2b).

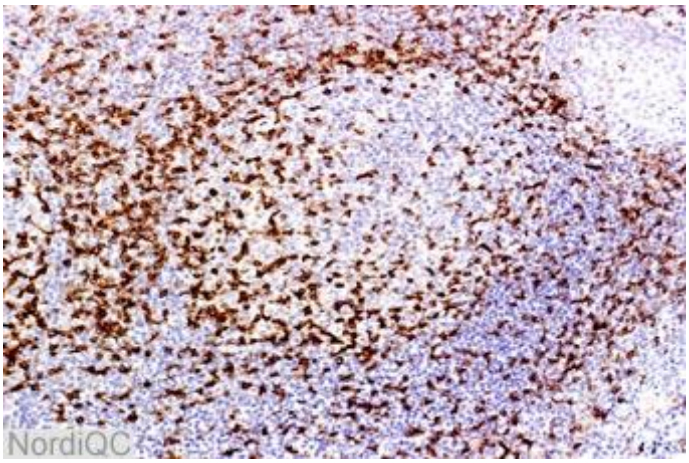

Fig. 4a
Staining for CD45 in the tonsil using an inappropriate antibody to CD45R0. Only the T-cells are demonstrated while the B-cells in germinal center and mantle zone are negative.

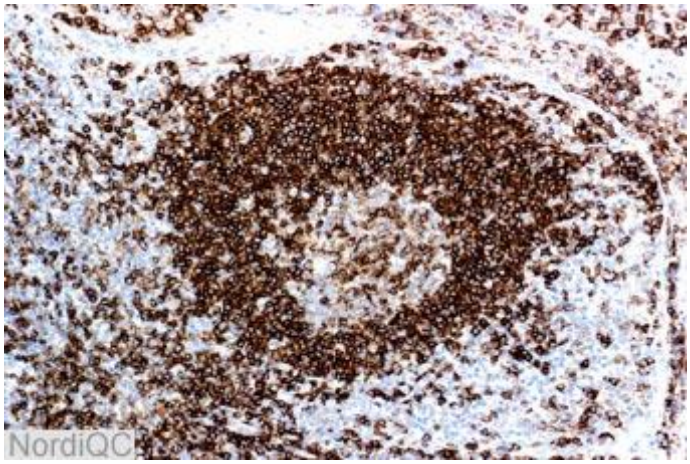

Fig. 4b
Staining for CD45 in the tonsil using an inappropriate antibody to CD45RA. The majority of B-cells are demonstrated while the T-cells are negative.

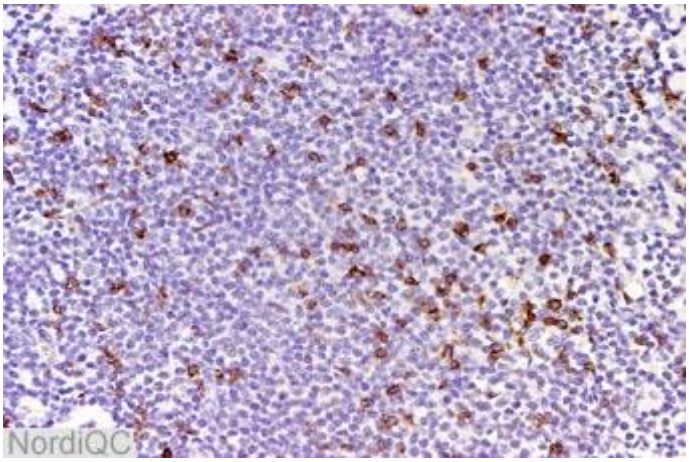

Fig. 5a
Staining for CD45 in the CLL using an inappropriate antibody to CD45R0. The neoplastic cells are negative and only the normal T-cells are stained.

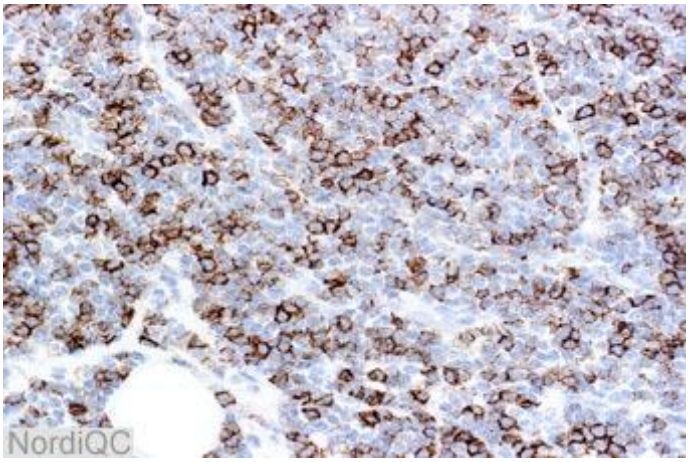

Fig. 5b
Staining for CD45 in the CLL using an inappropriate antibody to CD45RA. The majority of the neoplastic cells are stained. However, compare with Fig. 4b - the T-cells are not demonstrated. T-cell lymphomas will not be identified with CD45RA.

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