Assessment Run 17 2006

CD5

The slide to be stained for CD5 comprised:
1. Tonsil, 2 - 3. B-Chronic lymphatic leukaemia (B-CLL), 4. Peripheral T-cell lymphoma (NOS). 5 - 6. Mantle cell lymphoma. All specimens were fixed in 10 % NBF.

Criteria for assessing a CD5 staining as optimal included:

- A strong, distinct membranous staining of the majority of the normal peripheral T-cells in the tonsil.
- A strong, distinct membranous staining of majority of the neoplastic cells in the B-CLL and the mantle cell lymphoma.
- A strong, distinct membranous staining the residual normal T-cells in the T-cell lymphoma, whereas the neoplastic cells at least focally should be demonstrated.

88 laboratories submitted stains. At the assessment 33 achieved optimal marks (38 %), 25 good (28 %), 8 borderline (9 %) and 22 (25 %) poor marks.

The following Abs were used:
mAb clone **4C7** (Novocastra, n=55; NeoMarkers, n=6; Ventana, n=3; BioCare, n=1; Monosan, n=1)
mAb clone **CD5/54/F6** (Dako, n=14)
rmAb clone **SP19** (Ventana, n=5; NeoMarkers, n=3)

Optimal staining for CD5 in this assessment was obtained with the mAb clones **4C7** (28 out of 66) and **SP19** (5 out of 8). All optimal protocols were based on Heat Induced Epitope Retrieval (HIER).

With clone **4C7** all protocols giving an optimal staining were based on **HIER** with following buffers:
Tris-EDTA/EGTA pH 9 – 20/41 using this obtained an optimal mark,
CC1 (Cell Conditioning 1 - Ventana) - 5/8 laboratories using this obtained an optimal mark,
EDTA pH 8 – 2/4 using this obtained an optimal mark or
Citrate pH 6 - 1/4 using this obtained an optimal mark.
Clone **4C7** typically was used in the range of 1:25 – 1:400 depending on the total sensitivity of the protocol employed. 4C7 could also be used as an ready-to-use (RTU) Ab.

With clone **SP19** all protocols giving an optimal stain were based on **HIER** with either **CC1** (2 out of 5 laboratories using this obtained optimal marks) or **Tris-EDTA/EGTA pH 9** (2 out of 3 using this obtained optimal marks). With CC1 the optimal result was obtained by SP19 as RTU (2 out of 5, all Ventana) or diluted in the range of 1:50-1:100 (2 out of 3, all NeoMarkers).

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Excessive heat induced epitope retrieval

In the assessment almost all laboratories were able to demonstrate CD5 in the normal and neoplastic T-cells, whereas the prevalent feature of an insufficient staining was a too weak or false negative staining of the neoplastic B-cells in the two CLL and the two mantle cell lymphomas. In general, CD5 is only weakly expressed in B-cell lymphoma and a highly sensitive protocol is required to detect CD5 in B-cell lymphoma. Thus, normal T-cells will appear to be over-stained, but this has to be accepted in order to have a reliable method to demonstrate CD5 in B-cell lymphoma. Excessive HIER was another reason for insufficient staining as the morphology was impaired and - most critical - the fragile membranes of the neoplastic B-cells were extracted, resulting in a false negative CD5 reaction.

It should also be noticed that the most frequent cause of insufficient staining in this assessment seemed to be related to the choice of the primary Ab. 13 out of 14 laboratories using the mAb clone **CD5/54/F6** (93 %) were marked as insufficient – all using comparable setting as HIER in an alkaline buffer similar to the buffers applied for the clones **4C7** and **SP19**.

Normal tonsil can be used as positive control in which almost all peripheral T-cells should be stained as strongly as possible (with no reaction of germinal centre B-cells). A good quality indicator is the ability to demonstrate CD5 in a few peripheral mantle zone B-cells.

CD5 was also assessed in run 8 2003, in which 65 laboratories participated. Out of these, 23 laboratories (35 %) had an insufficient staining. Each laboratory was given specific recommendations to improve their protocol. 20 laboratories, which obtained an insufficient result in run 8 submitted a new CD5 stain in run 17. 16 out of these followed the recommendation, and 10 of them (63 %) improved from insufficient to Sufficient (good or optimal). 4 laboratories did not follow the recommendations and only one (25 %) improved from insufficient to sufficient (good).

The overall proportion of insufficient staining was in this run almost identical (34 %) to that of run 8 (35 %). Focusing only on the laboratories participating in both runs (n=58), the proportion of insufficient staining was only slightly reduced reduced from 35 % (n=20) to 29 % (n=17).

Conclusion
The mAb clones **4C7** and **SP19** are useful for the detection of CD5 in both T-cell and B-cell lymphomas. All 8 laboratories using clone **SP19** were either marked good or optimal and 49 out of 66 (74 %) using clone **4C7** were marked as good or optimal. HIER in an alkaline buffer as Tris-EDTA/EGTA pH 9 or CC1 is highly recommended for optimal performance.

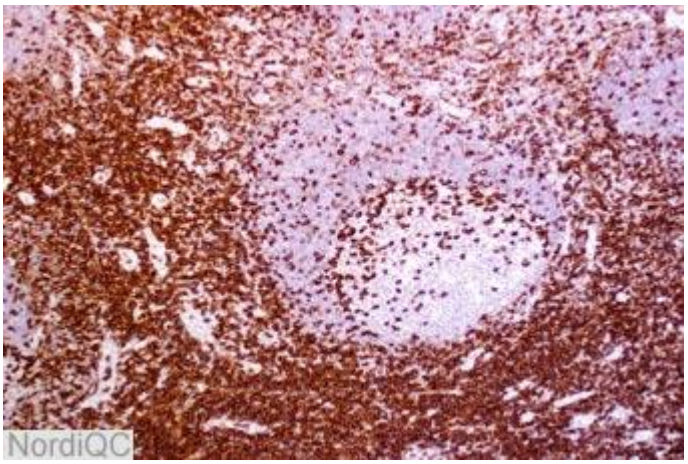

Fig. 1a
Optimal staining for CD5 of the tonsil. The clusters of T-cells in the inter-follicular areas are strongly stained and also the isolated T-cells within the secondary lymphoid follicles are demonstrated.

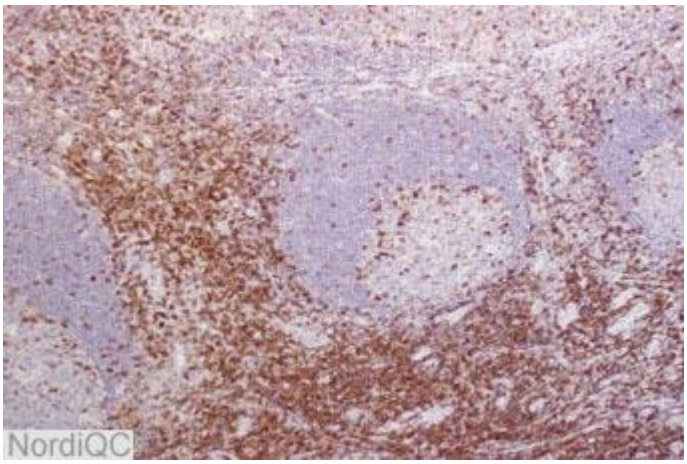

Fig. 1b
Staining for CD5 of the tonsil using an insufficient protocol (same field as in Fig. 1a.). The cells expected to cells are demonstrated. However, compare with Fig. 3b – same protocol.

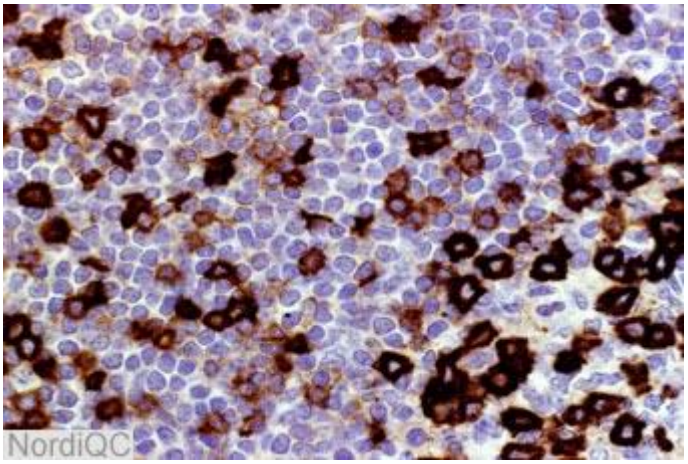

Fig. 2a
High magnification of the mantle zone and germinal centre in Fig 1a. The T-cells in the edge of the germinal centre and in the mantle zone show a strong staining. In between the strongly stained T-cells in the mantle zone, a population of cells (B-cells) shows a moderate membranous reaction.

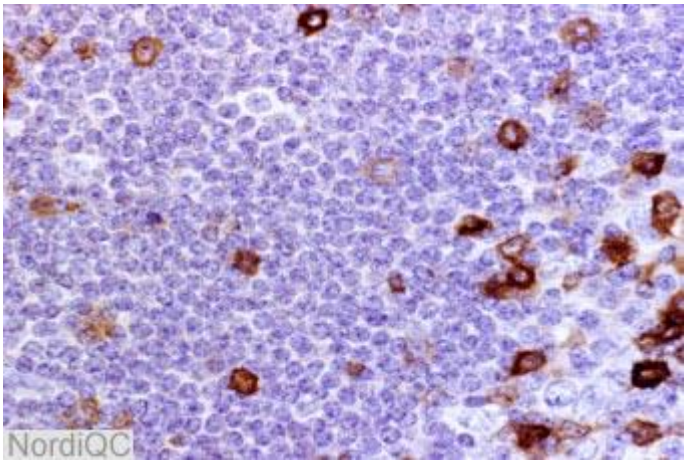

Fig. 2b
High magnification of the mantle zone and germinal centre in Fig 1b. The proportion of positive T-cells is reduced compared to the result in Fig 2a. More important, the population of mantle cell B-cells are virtually negative.

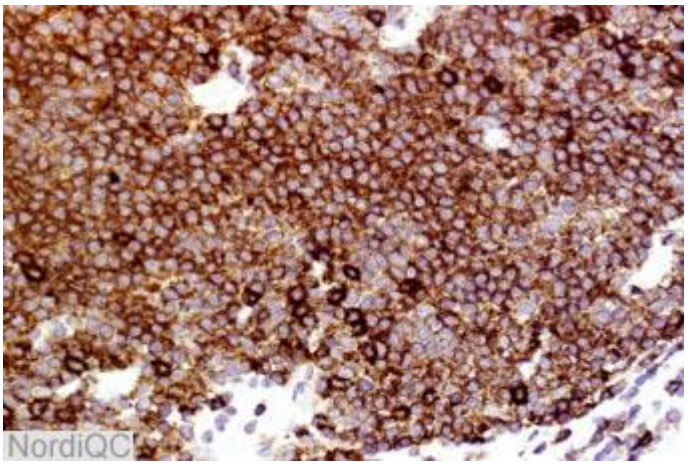

Fig. 3a
Optimal staining for C5 of the mantle zone lymphoma. The majority of the neoplastic cells show a moderate and distinct staining. The entrapped normal T-cells show a strong staining (same protocol as in Fig. 1a and 2a).

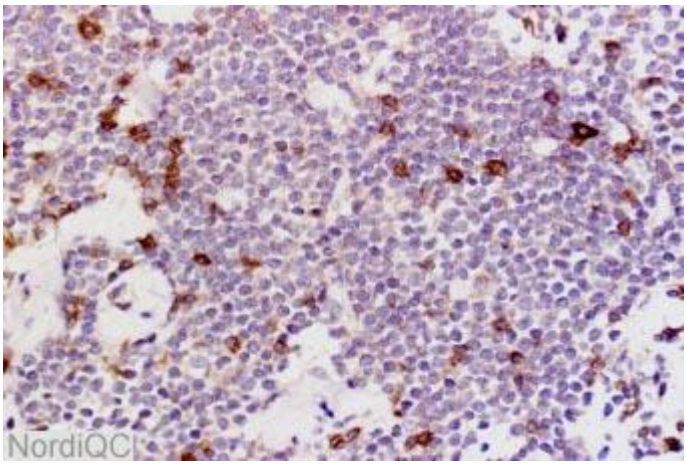

Fig. 3b
Insufficient staining for C5 of the mantle zone lymphoma (same field as in Fig. 3a). The neoplastic cells are negative and only the normal T-cells are demonstrated (same protocol as in Fig. 1b and 2b).

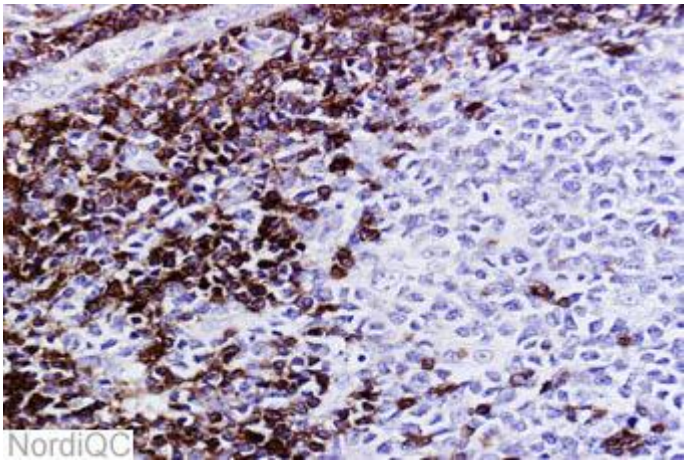

Fig. 4a
Insufficient staining for CD5 in the tonsil. The germinal centre B-cells show an impaired morphology and the T-cells show an imprecise localization of CD5, most likely due to excessive heat induced epitope retrieval. Almost the same protocol as used in Fig. 1a-3a, but with a too harsh epitope retrieval.

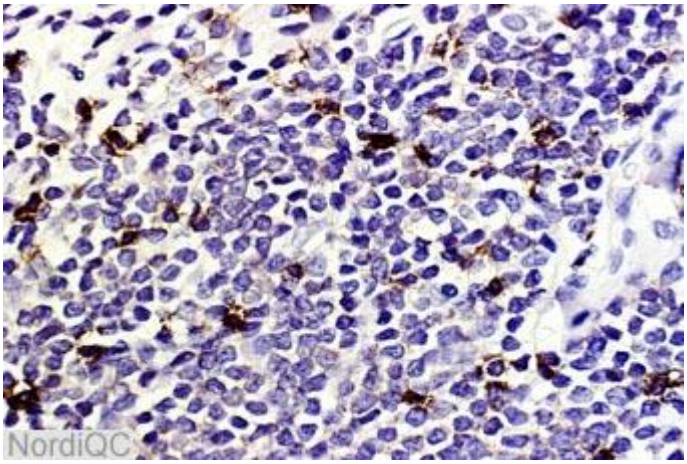

Fig. 4b
Insufficient staining for C5 of the mantle zone lymphoma (same protocol as in Fig. 4a). All the neoplastic cells are virtually negative as the cellular membranes are extracted due to the excessive heat induced epitope retrieval.

SN/MV/LE 26-6-2006