Assessment Run 24 2008

CD5

The slide to be stained for CD5 comprised:
1. Tonsil fixed 24 h, 2. Tonsil fixed 72 h, 3-4. B-Chronic lymphatic leukaemia (B-CLL), 5. Mantle cell lymphoma (MCL).
All tissues were fixed in 10% neutral buffered formalin.

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 tonsils. Both dispersed and grouped T-cells should be distinctively demonstrated.
- An at least weak to moderate membranous staining of a few B-cells in the mantle zone of the secondary follicles in the tonsils.
- A moderate to strong, distinct membranous staining of majority of the neoplastic cells in the two B-CLLs and the MCL.

119 laboratories submitted stains. At the assessment 52 achieved optimal marks (44 %), 28 good (24 %), 16 borderline (13 %) and 23 poor marks (19 %).

The following Abs were used:
mAb clone **4C7** (Novocastra/Leica, n=69; NeoMarkers/Thermo, n=6; Monosan, n=4; BioCare, n=2; VECTOR, n=1)
mAb clone **CD5/54/F6** (Dako, n=14)
rmAb clone **SP19** (Ventana, n=11; NeoMarkers/Thermo, n=8; Dako, n=2)
pAb **E2474** (Spring Bioscience, n=1)
NS n=1

Optimal staining for CD5 in this assessment was obtained with the mAb **4C7** (40 out of 82; 49%) and the rmAb **SP19** (6 out of 21; 26%).

All optimal protocols, independent of the Ab were based on heat induced epitope retrieval (HIER) using following HIER buffers and protocol settings:

4C7: Tris-EDTA/EGTA pH 9.0 (23/42)*, Cell Conditioning1 (BenchMark, Ventana) (2/9), Target Retrieval Buffer pH 9, (Dako) (6/7), Bond Epitope Retrieval Solution 2 (Bond, Leica) (5/9), EDTA/EGTA pH 8 (3/3), Citrate pH 6.0 (3/4) or Target Retrieval Solution pH 6.1 (Dako) (1/2). The mAb was diluted in the range of 1:20 – 1:300 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) Ab. Using these protocol settings 59 out of 78 (76 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

SP19: Target Retrieval Buffer pH 9, (Dako) (4/4), Cell Conditioning1 (BenchMark, Ventana) (1/13) and Tris-EDTA/EGTA pH 9.0 (1/3). The rmAb was diluted in the range of 1:25 – 1:75 depending on the total sensitivity of the protocol employed or as a Ready-To-Use Ab.
Using these protocol settings 16 out of 20 (80 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Less successful primary antibody (all of 14 protocols based on the clone CD5/54/F6 gave an insufficient result).
- Too low concentration of the primary antibody
- Insufficient HIER (espc. non-alkaline buffer such as Citrate pH 6.0, and/or too short HIER)

In this assessment and in concordance to the previous assessment of CD5 in run 17 almost all laboratories were able to demonstrate CD5 in the normal T-cells in both the tonsils and the lymphomas, 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 MCL. In general, CD5 is only weakly expressed in B-cell lymphoma and a highly sensitive protocol is required to detect CD5 in this tumour. It was observed that the clone CD5/54/F6 gave an acceptable reaction in the normal T-cells, but in all of 14 protocols using this clone the reaction was insufficient in the B-cell lymphomas

and in particular in the mantle cell lymphoma, despite otherwise optimal protocol settings. Comparing the Abs used in the last 2 assessments for CD5 listed in table 1, the data indicate that the clone CD5/54/F6 can not be recommended as a marker for CD5 in lymphoma classification.

Table 1

	Run 17 & 24 All protocol settings			**Run 17 & 24 Optimal protocol settings***		
	Protocols	**Sufficient**	**Optimal**	**Protocols**	**Sufficient**	**Optimal**
mAb clone 4C7	148	107 (72%)	71 (48%)	145	107 (74%)	71 (49%)
mAb clone CD5/54/F6	28	1 (4%)	0 (0%)	28	1 (4%)	0 (0%)
rmAb SP19 conc.	11	10 (91%)	5 (46%)	11	10 (91%)	5 (46%)
rmAb SP19 RTU VMS	16	11 (69%)	2 (13%)	14	11 (79%)	2 (14%)
rmAb SP19 RTU Dako	3	3 (100%)	3 (100%)	3	3 (100%)	3 (100%)

*Appropriate HIER and dilution of the antibody (listed for each marker in the 2 assessments).

It was observed that normal tonsil can be used as positive control in which virtually all the peripheral T-cells should be stained as strongly as possible (with no reaction of germinal centre B-cells). A recommendable critical staining quality indicator was the ability to demonstrate CD5 in a few peripheral mantle zone B-cells. The staining reaction for CD5 in the tonsils fixed in 10 % NBF for 24 and 72 hours respectively was identical.

This was the third assessment of CD5 and the proportion of sufficient results has been almost constant in three runs, which is shown in table 2 below:

Table 2

	Run 8 2003	Run 17 2006	Run 24 2008
Participants, n=	65	88	119
Sufficient results	65%	66%	68%

56 laboratories participating in all 3 runs for CD5 showed an increase in the proportion of sufficient results from 61 % (run 8) to 75 % (run 24). The relative low proportion of sufficient results and lack of improvement of the pass rate in the 3 runs seem to be related to the constant increase in the number of participants as well as the persistent usage of the mAb clone CD5/54/F6.

CD5 was also assessed in **run 17 2006**, in which 88 laboratories participated. Out of these, 30 laboratories (34 %) had an insufficient staining. Each laboratory was given specific recommendations to improve their protocol. 28 laboratories, which obtained an insufficient result in run 17, submitted a new CD5 stain in run 24. 17 out of these followed the recommendation, and 15 (88 %) improved from insufficient to sufficient (good or optimal). 9 laboratories did not follow the recommendations and only one (11 %) improved from insufficient to sufficient (good). 2 laboratories changed their entire IHC system and both improved to sufficient.

Conclusion

The mAb clone **4C7** and the rmAb **SP19** are both useful markers for the detection of CD5 in B-cell lymphomas. HIER is mandatory and an alkaline buffer is highly recommended for optimal performance. Normal tonsil is a recommendable control providing a few peripheral mantle zone B-cells are demonstrated and show an at least weak to moderate membranous staining. The mAb clone CD5/54/F6 should not be used.

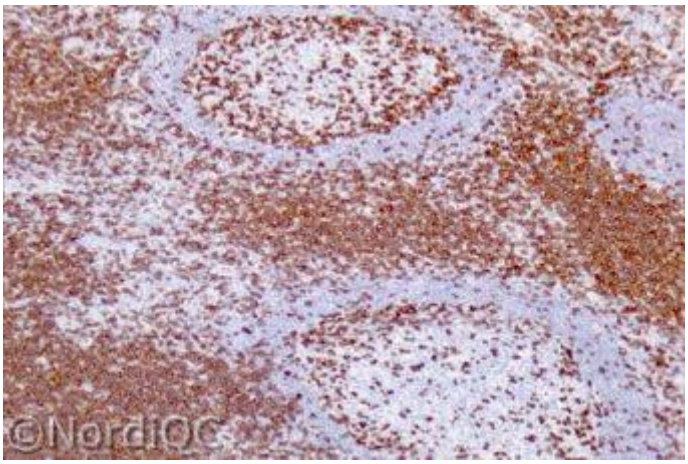

Fig. 1a
Optimal staining for CD5 of the tonsil (X100) using the rmAb clone SP19, optimally calibrated and with HIER. The clusters of T-cells in the inter-follicular areas are strongly stained and also the scattered T-cells within the secondary lymphoid follicles are demonstrated.

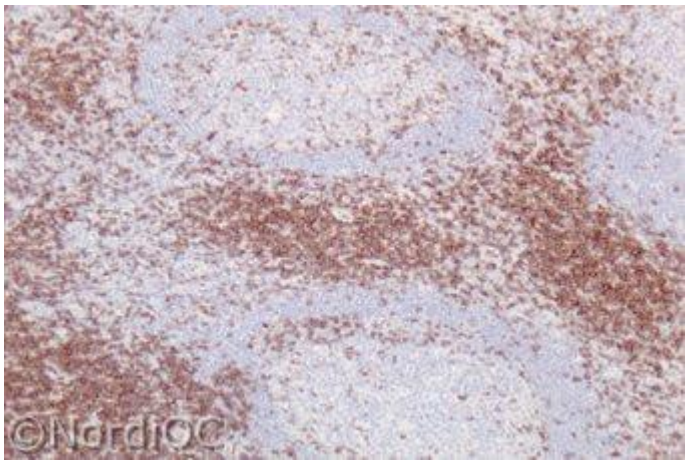

Fig. 1b
Staining for CD5 of the tonsil (same field as in Fig. 1a.) using an insufficient protocol based on the mAb clone CD5/54/F6 with HIER. The majority of T-cells expected to be stained are demonstrated, however compare with Fig. 3b & 4b – same protocol.

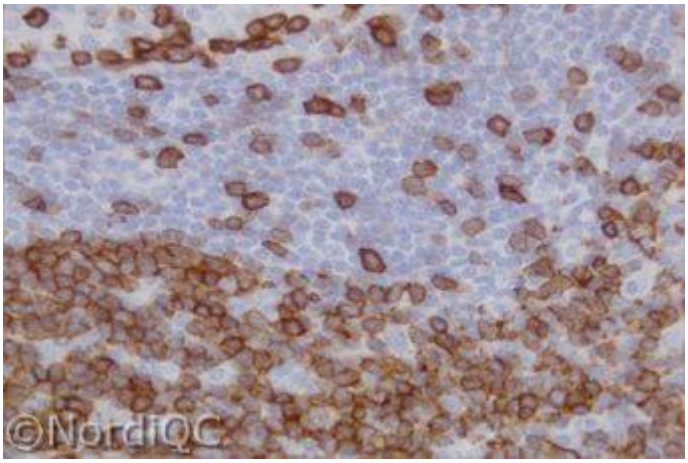

Fig. 2a
High magnification (x400) of the mantle zone and germinal centre in Fig. 1a. The T-cells at the edge of the germinal centre and in the mantle zone show a strong and intense staining. In between the strongly stained T-cells in the mantle zone a population of cells (B-cells) shows a moderate but distinct membranous reaction.

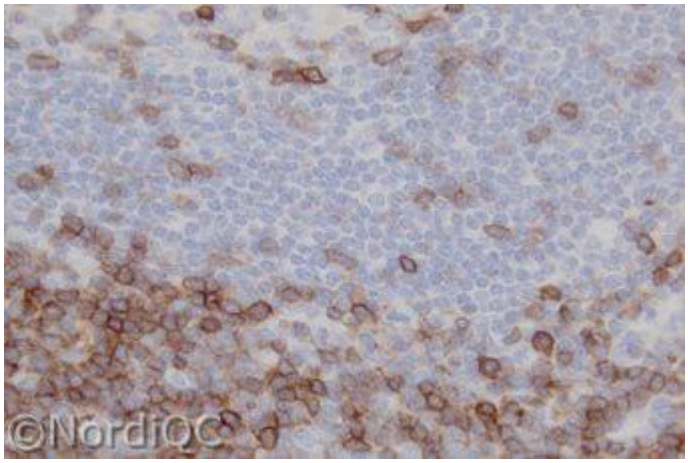

Fig. 2b
High magnification (x400) of the mantle zone and germinal centre in Fig. 1b. The proportion and intensity of positive T-cells is reduced compared to the result in Fig. 2a, but more important is that the population of mantle cell B-cells is virtually negative.

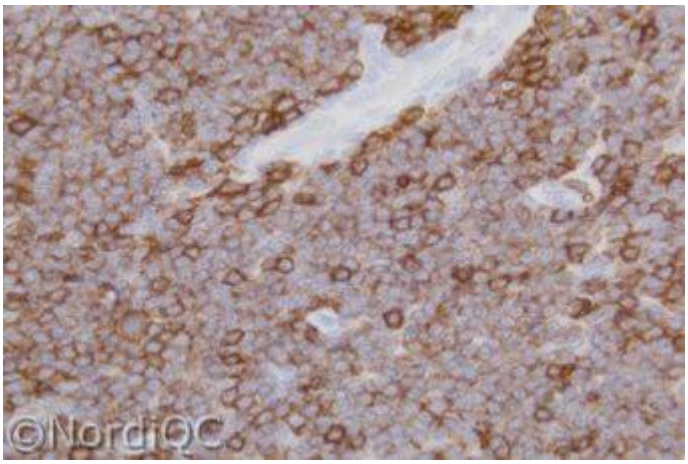

Fig. 3a
Optimal staining for CD5 of the B-CLL using same protocol as in Fig. 1a & 2a. The majority of the neoplastic cells show a moderate and distinct staining. The entrapped normal T-cells show a strong staining.

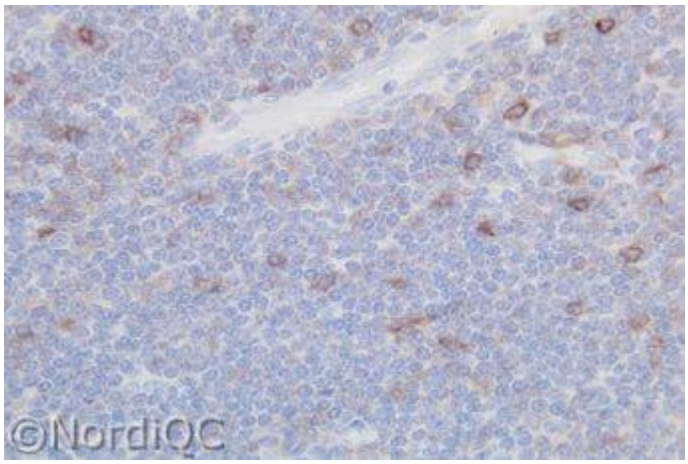

Fig. 3b
Insufficient staining for CD5 of the B-CLL using same protocol as in Fig. 1b & 2b (same field as in Fig. 3a). The neoplastic cells are almost negative and only the normal T-cells are clearly demonstrated.

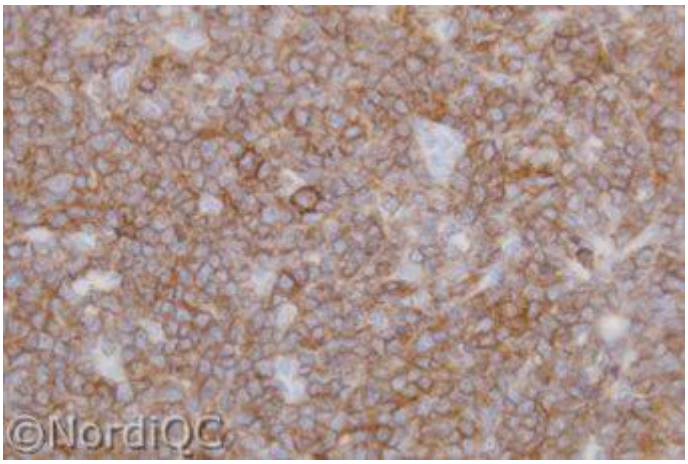

Fig. 4a
Optimal staining for CD5 of the mantle lymphoma using same protocol as in Fig. 1a - 3a. The majority of the neoplastic cells show a moderate and distinct staining.

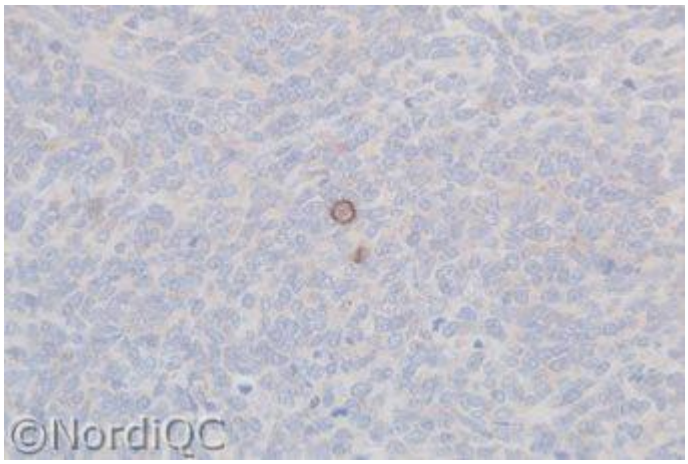

Fig. 4b
Insufficient staining for CD5 of the mantle lymphoma using same protocol as in Fig. 1b - 3b (same field as in Fig. 4a). The neoplastic cells are negative and only the normal T-cells are demonstrated.

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