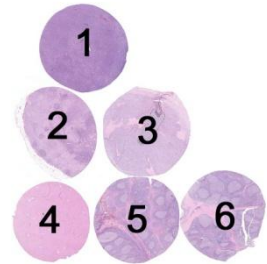# Assessment Run 29 2010
CD4

The slide to be stained for CD4 comprised:
1. B-cell chronic lymphatic leukaemia (B-CLL) with transformation to classic Hodgkin Lymphoma, 2. T-cell lymphoma (Sezary syndrome), 3. T-cell lymphoma (NOS), 4. Liver, 5. Tonsil fixed 24 h, 6. Tonsil fixed 72 h.
All tissues were fixed in 10 % neutral buffered formalin.

Criteria for assessing a CD4 staining as optimal included:

- A strong, distinct, predominantly membranous staining of the normal helper/inducer T-cells in both the T-zone and within the germinal centres in the tonsils.
- An at least moderate, distinct staining of macrophages, in particular germinal centre macrophages in the tonsils, and Kupffer cells and endothelial cells in the liver sinusoids.
- A strong, distinct, predominantly membranous reaction of the neoplastic cells in the CD4 positive T-cell lymphoma, Sezary syndrome.
- A strong, distinct, predominantly membranous staining of the normal helper/inducer T-cells and moderate staining of macrophages in the B-CLL with transformation to classic Hodgkin Lymphoma.
- No staining of other cells. Especially the B-cells and Hodgkin/Reed-Sternberg cells should be negative.

129 laboratories participated in the assessment. 63 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD4, run 29**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4B12**	33 14 7 5 1 1	Leica/Novocastra NeoMarkers Dako Monosan Master Diagnostica Vector	16	21	9	15	61 %	74 %
mAb clone **1F6**	28 2	Leica/Novocastra BioCare	6	7	7	10	43 %	50 %
rmAb clone **SP35**	3 1	Spring Bioscience Immunologic	3	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **4B12, IR649**	14	Dako	9	2	2	1	79 %	100 %
mAb clone **4B12, PA0368**	4	Leica/Novocastra	0	1	0	3	-	-
mAb clone **1F6, PM153**	1	BioCare	1	0	0	0	-	-
mAb clone **1F6, MONX10330**	1	Monosan	0	1	0	0	-	-
rmAb clone **SP35, 104R/790-4423**	14	Cell Marque/Ventana	11	2	1	0	93 %	100 %
Total	129		46	35	19	29	-	
Proportion			36 %	27 %	15 %	22 %	64 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

Conc. Abs
mAb clone **4B12**: the protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/13)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (9/26), Bond Epitope Retrieval

Solution 2 (Bond, Leica) (2/9), Borg pH 9 (BioCare) (1/1), EDTA/EGTA pH 8 (1/4) or Citrate pH 6 (2/5) as the retrieval buffer. The mAb was typically diluted in the range of 1:30– 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 31 out of 42 (74 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **1F6**: the protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (1/5), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (1/6), Bond Epitope Retrieval Solution 2 (Bond, Leica) (3/3) or Cell Conditioning 1 (BenchMark, Ventana) (1/13) as the retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 10 out of 20 (50 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **SP35**: the protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using Cell Conditioning 1 (BenchMark, Ventana) (2/2) as the retrieval buffer. The mAb was diluted in the range of 1:25– 1:1:75. Using these protocol settings all of 3 laboratories produced an optimal staining.

Ready-To-Use Abs
mAb clone **4B12** (prod. no IR649, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab (1 lab used 40 min) and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 11 (100 %) laboratories produced a sufficient staining.

mAb clone **1F6** (prod. no PM153, BioCare): The protocol giving an optimal result was based on HIER in a pressure cooker using Borg pH 9 (BioCare), an incubation time of 30 min in the primary Ab and MACH 3 Mouse HRP Polymer as the detection system.

rmAb clone **SP35** (prod.no. 104R/790-4423, Cell Marque/Ventana): The protocols giving an optimal result were all based on HIER using mild or standard Cell Conditioning 1, an incubation time of 16-44 min in the primary Ab and UltraView (760-500) as the detection system. Using these protocol settings all of 13 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were (often in combination):

- Too low concentration of the primary antibody
- Insufficient HIER
- Less successful performance of the mAb clones 4B12 and 1F6 on the Ventana BenchMark platform.

In this assessment and in concordance to the previous assessment run 14, 2005, the prevalent feature of an insufficient staining was a generally too weak or completely false negative staining reaction of the cells expected to stain. This pattern was seen in both the normal helper/inducer T-cells, the endothelial cells of the liver sinusoids and the neoplastic T-cells of the Sezary syndrome. The newly launched rmAb clone SP35 was in this assessment the most robust and successful Ab, as 17 out of 18 laboratories (94%) using this clone obtained a sufficient mark, in contrast to the well established mAb clones 1F6 and 4B12, where a sufficient mark only was obtained in 15 out 32 laboratories (47%) and 49 out of 79 laboratories (62%), respectively.

In the previous run 14 (2005) it was found that the antigen detected by the mAb clone 1F6 is deteriorated by blocking of endogenous peroxidase in > 1 % H_2O_2 after HIER. In order to monitor this, NordiQC asked the participants both to state the concentration of H_2O_2 used and information whether the blocking step was performed before or after HIER. Unfortunately the protocol template used for this information was out of order and NordiQC did not obtain reliable data.

The pass rates were influenced by the stainer platform used. When the mAb clone 4B12, as a concentrate, was used on the Leica Bond-max™ platform, 10 out of 11 protocols (91%) gave a sufficient result, whereas all 9 protocols based on the same clone and similar protocol settings applied on the Ventana BenchMark gave an insufficient result (8 assessed as poor). The reason for this discrepancy is currently not known.

In this assessment the Dako RTU system for the mAb clone 4B12 was very successful as all of 11 stains based on this platform according to the recommendations from Dako were assessed as sufficient (9 were optimal). For the Ventana BenchMark platform the RTU format of the rmAb clone SP35, performed well according to the recommendations from Ventana/Cell Marque, a sufficient result in all of 13 stains was obtained (11 were optimal).
This was the 2nd assessment of CD4 in NordiQC. The proportion of sufficient results decreased from 73 % in run 14, 2005, to 63 % in the current run. The lower pass rate may be due to several factors (new tissue material

circulated, many new participants).

Table 2. **Proportion of sufficient results for CD4 in the two NordiQC runs performed**

	Run 14 2005	Run 29 2010
Participants, n=	59	129
Sufficient results	72 %	63 %

As observed in run 14 tonsil is a recommendable control for CD4 as the germinal centre macrophages were found to be a reliable stain quality indicator: These cells typically were demonstrated in a staining assessed as optimal or good, while the cells were too weak or negative in a staining assessed as borderline or poor.

Conclusion
The mAb clones 1F6, 4B12 and the rmAb clone SP35 are all recommendable Abs for CD4. The performances of the Abs seem to be influenced by the stainer platform. HIER, preferable in an alkaline buffer, is mandatory for optimal performance. Tonsil is a recommendable positive control: The helper/inducer T-cells must show a strong membranous staining and the germinal centre macrophages an at least weak to moderate, distinct staining.
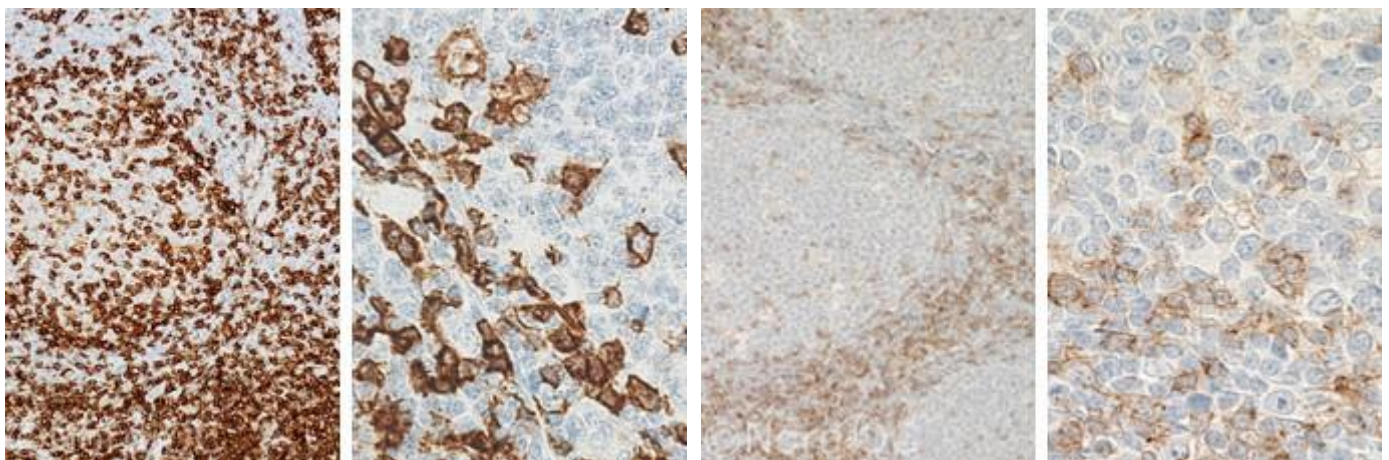

Fig. 1a
Optimal CD4 staining of the tonsil using the rmAb clone SP35 with HIER in an alkaline buffer.
Left: The majority of the T-cells in the T-zone and in the germinal centre show a strong membranous staining, while the germinal centre macrophages show a weak to moderate but distinct membranous staining.
Right: High magnification of the edge of the germinal centre showing both the T-cells and the germinal centre macrophages.

Fig. 1b
Insufficient CD4 staining of the tonsil, using the mAb clone 1F6 with protocol settings giving a too low sensitivity.
Left: The T-cells are demonstrated, but the number and intensity is significantly reduced as obtained by the protocol used in Fig. 1a.
Right: High magnification of the edge of the germinal centre. Only the T-cells show a weak membranous staining, while the macrophages are negative. Also compare with Figs. 2b & 3b – same protocol.
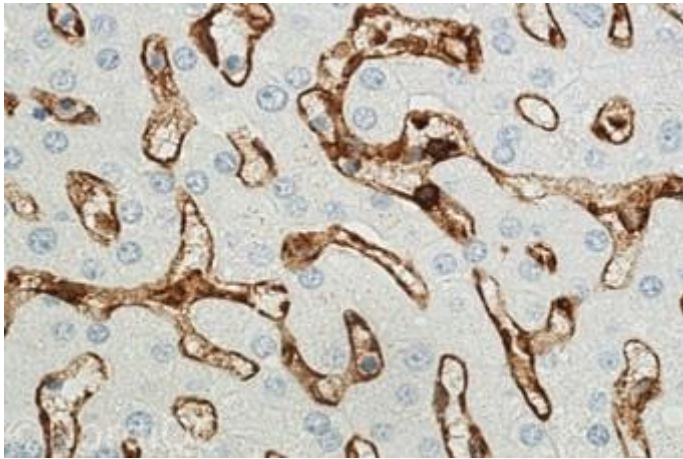
Fig. 2a
Optimal CD4 staining of the liver using same protocol as in Fig. 1a. Both the endothelial cells lining the sinusoids and the Kupffer cells show a moderate to strong predominantly membranous staining, while the liver cells are unstained.
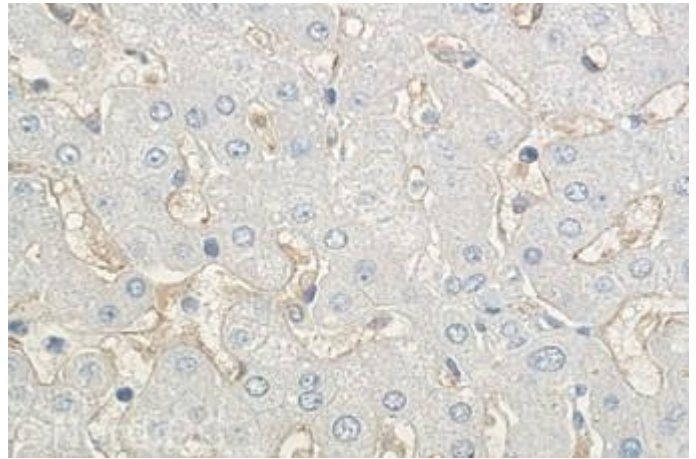

Fig. 2b
Insufficient CD4 staining of the liver using same protocol as in Fig. 1b. The endothelial cells and the Kupffer cells show only a weak and equivocal staining. Compare with Fig. 3b - same protocol.
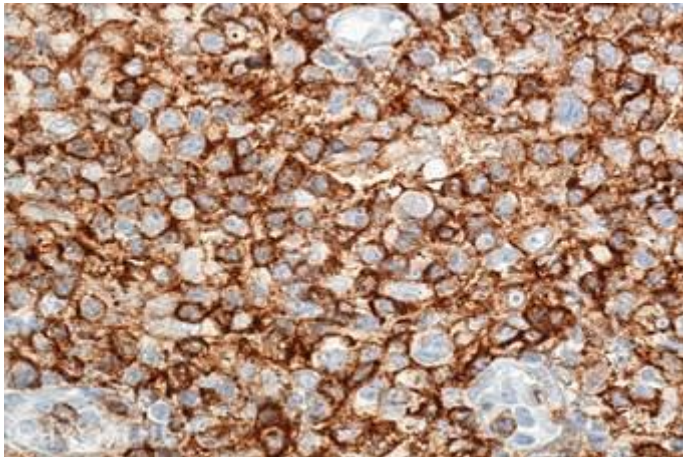

Fig. 3a
Optimal CD4 staining of the CD4 positive T-cell lymphoma (Sèzary syndrome) no. 2 in the multitissue block using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a distinct membranous staining.
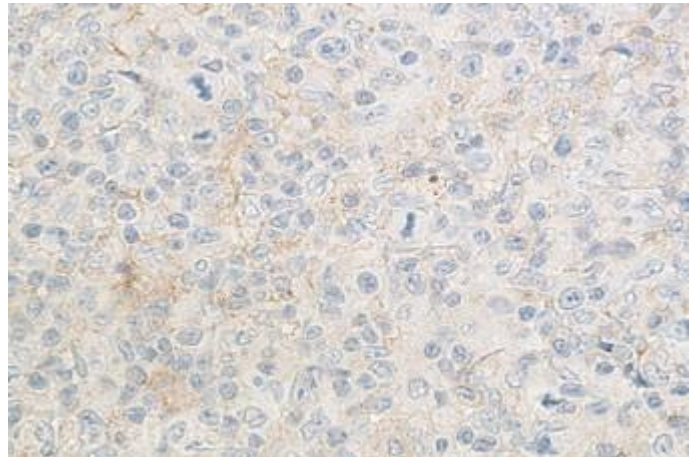

Fig. 3b
Insufficient CD4 staining of the CD4 positive T-cell lymphoma (Sèzary syndrome) no. 2 in the multitissue block using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show an equivocal staining.
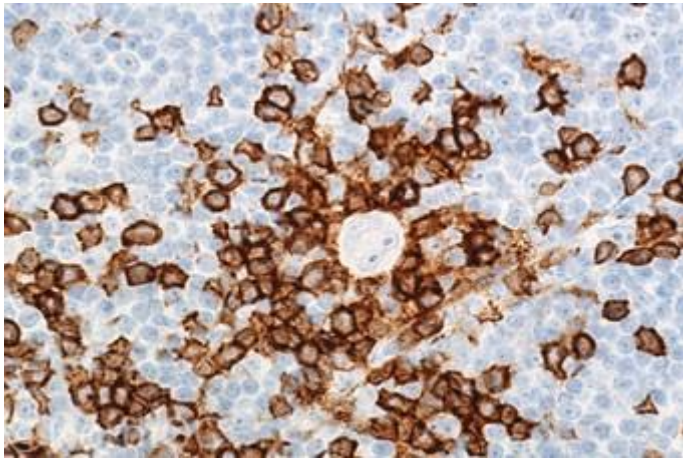
Fig. 4a
Optimal CD4 staining the B-CLL transforming to classic Hodgkin lymphoma using same protocol as in Figs. 1a, 2a & 3a. The neoplastic cells including Reed-Sternberg cells are unstained, while the T-cells and macrophages show a distinct membranous staining.
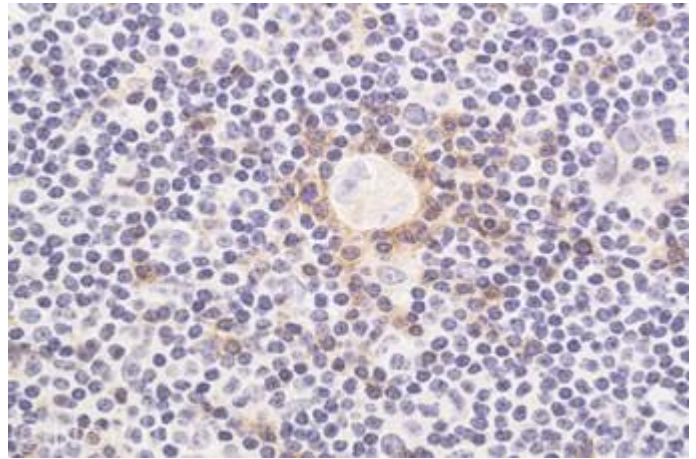

Fig. 4b
Insufficient CD4 staining of the B-CLL transforming to classic Hodgkin lymphoma using same protocol as in Figs. 1b, 2b & 3b. The neoplastic cells including Reed-Sternberg cells are unstained while T-cells are stained weakly but macrophages are virtually unstained.

SN/MV/LE 2-7-2010