Assessment Run 22 2008
CD3

The slide to be stained for CD3 comprised:
1. Colon, 2. Tonsil, 3. Precursor T-cell lymphoma, 4-5. Peripheral T-cell lymphomas.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD3 staining as optimal included:

- A strong and distinct predominantly membranous reaction of the majority of neoplastic T-cells in all 3 T-cell lymphomas.
- A strong and distinct predominantly membranous staining of all the T-cells in the tonsil and in the colon.
- No staining in other cells. Especially B-cells in the tonsil should be negative.

119 laboratories submitted stains. At the assessment 64 achieved optimal marks (54 %), 35 good (30 %), 16 borderline (13 %) and 4 poor marks (3 %).

The following Abs were used:
mAb clone **PS1** (Novocastra, n=26; BioCare Medical, n=2; Monosan, n=1; Vector, n=1)
mAb clone **F7.2.38** (Dako, n=28)
mAb clone **LN10** (Novocastra, n=6)
rmAb clone **SP7** (NeoMarkers, n=17; DCS, n=1; Master Diagnostica, n=1)
rmAb clone **2GV6** (Ventana, n=7)
rmAb clone **E272** (BioCare Medical, n=1)
pAb **A0452** (Dako, n=20)
pAb **760-2613** (Ventana, n=5)
pAb **NCL-CD3p** (Novocastra, n=2)
pAb **RB-360** (NeoMarkers, n=1)

Optimal staining for CD3 in this assessment was obtained with the mAbs clone **PS1** (19/30)*, clone **F7.2.38** (15/28), the mAb clone **LN10** (4/6), the rmAb clone **SP7** (7/19), the rmAb clone **2GV6** (7/7) and the pAb **A0452** (12/20).
* (number of optimal results/number of laboratories using this Ab).

PS1: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (14/17)**, Cell Conditioning1 (BenchMark, Ventana) (2/7), Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems)(2/3) or EDTA/EGTA pH 8 (1/1). The mAb was typically diluted in the range of 1:20 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 24 out of 28 (86 %) laboratories produced a sufficient staining (i.e., optimal or good).
** (number of optimal results/number of laboratories using this buffer).

F7.2.38: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (15/21). The mAb was diluted in the range of 1:50 – 1:400 depending on the total sensitivity of the protocol employed or as a Ready-To-Use antibody. Using these protocol settings 19 out of 19 (100 %) laboratories produced a sufficient staining.

LN10: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (2/2) or Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) (2/2). The mAb was typically diluted in the range of 1:100 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining.

SP7: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (5/6), Cell Conditioning1 (BenchMark, Ventana) (1/6) or Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems)(1/1). The rmAb was typically diluted in the range of 1:40 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 out of 10 (90 %) laboratories produced a sufficient staining.

2GV6: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Cell Conditioning1 (BenchMark, Ventana) (6/6) or EDTA/EGTA pH8 (1/1). The rmAb was used as a Ready-To-Use antibody. Using these protocol settings 7 out of 7 (100 %) laboratories produced a sufficient staining (all marked as optimal).

A0452: the protocols giving an optimal result was all based on HIER using Tris-EDTA/EGTA pH 9 (5/10), Cell Conditioning1 (BenchMark, Ventana) (3/3), Target Retrieval Solution pH 9 (FLEX TRS high pH, Dako) (3/3) or EDTA/EGTA pH8 (1/1). The pAb was diluted in the range of 1:50– 1:500 depending on the total sensitivity of the protocol employed or applied as a Ready-To-Use antibody. Using these protocol settings 15 out of 16 (94 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient HIER (espc. non-alkaline buffer as Citrate pH 6.0)
- Less successful primary antibody.

In this assessment the prevalent feature of an insufficient result was a too weak or false negative staining reaction in both the normal and neoplastic T-cells. Virtually all laboratories could sufficiently demonstrate CD3 in the interfollicular T-cells in the tonsil, whereas a generally too weak or false negative reaction was seen in the dispersed germinal centre T-cells and the intraepithelial T-cells in the colon.
It was not possible to differentiate between the cytoplasmic CD3 expression in the precursor T-cell lymphoma and the membranous CD3 expression in the peripheral T-cell lymphomas, irrespective of the Ab or protocol used. Tonsil is a recommendable control for CD3: The dispersed germinal centre and mantle zone T-cells must show a strong and distinct staining without reaction of the B-cells.

This was the third assessment of CD3. A constant increase in the proportion of sufficient results has been seen, as shown in table:

	Run 5 2001	Run 14 2005	Run 22 2008
Participants, n=	47	87	119
Sufficient results	69%	73%	84%

The availability of several robust Abs for CD3 combined with efficient HIER seems to be the main reason for the high pass rate. HIER in Citrate pH 6,0 was found to be inferior to an alkaline HIER buffer irrespective of the antibody applied, as an optimal result could not be obtained using Citrate and 4 out of the 10 protocols based on HIER in Citrate gave an insufficient result. The increase in the proportion of sufficient results has been accomplished despite a large number of new participants. The tailored recommendations to the laboratories with insufficient staining results seem to be useful: In run 14, 22 laboratories obtaining an insufficient mark were given a specific recommendation to adjust their protocol for CD3 and submitted a new stain in run 22. 15 laboratories followed the recommendation and 14 of these (93%) improved their result. 3 did not follow the recommendations and 1 of these (33%) improved. 4 laboratories changed their entire system obtaining a sufficient result.

Conclusion
The mAbs clones PS1, F7.2.38, LN10, the rmAbs SP7, 2GV6 and the pAb A0452 are all useful markers for CD3. HIER in an alkaline buffer seems to be mandatory for an optimal CD3 staining.

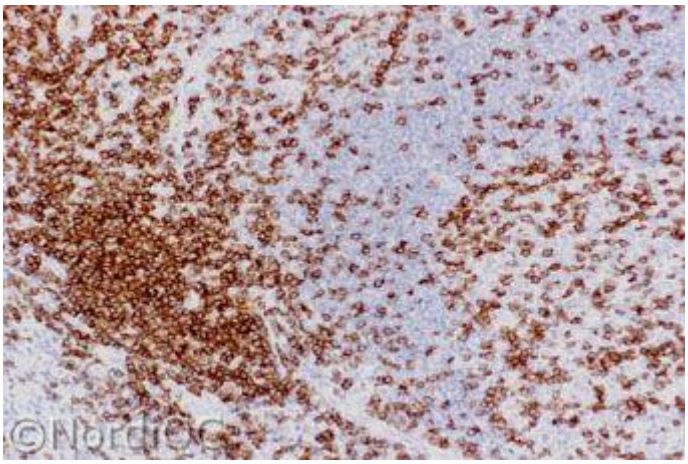

Fig. 1a
Optimal CD3 staining of the tonsil using the mAb clone PS1. Virtually all the peripheral T-cells, both the crowded T-cells in the interfollicular T-zone and the dispersed T-cells in the germinal centre show a strong and distinct staining.

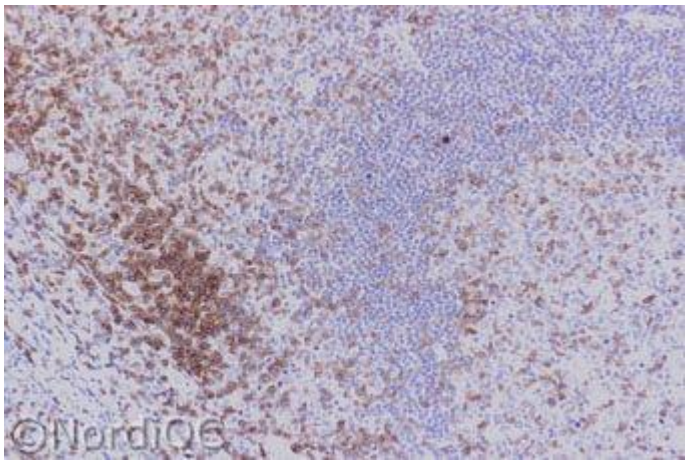

Fig. 1b
CD20 staining of the tonsil – same field as in Fig. 1a – using an insufficient protocol based on clone PS1 (too low concentration of the Ab). Only the crowde7337d T-cells in the T-zone are distinctively demonstrated, whereas the dispersed T-cells only show a weak reaction. Also compare with Fig. 2b and 3b – same protocol.

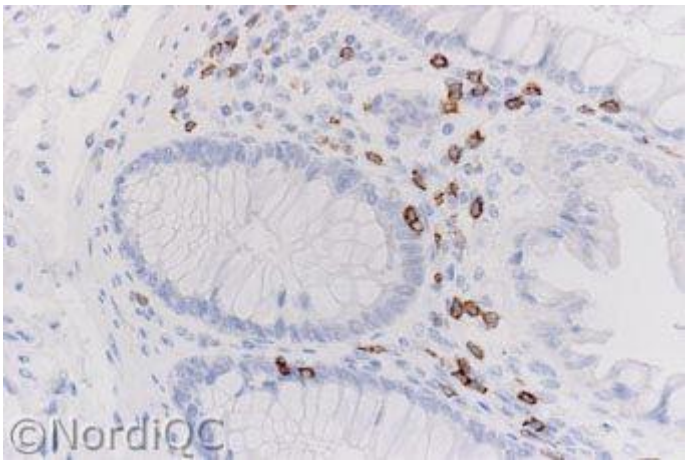

Fig. 2a
Optimal CD3 staining of the colon using same protocol as in Fig. 1a. Both inter- and intra-epithelial T-cells show a distinct staining.

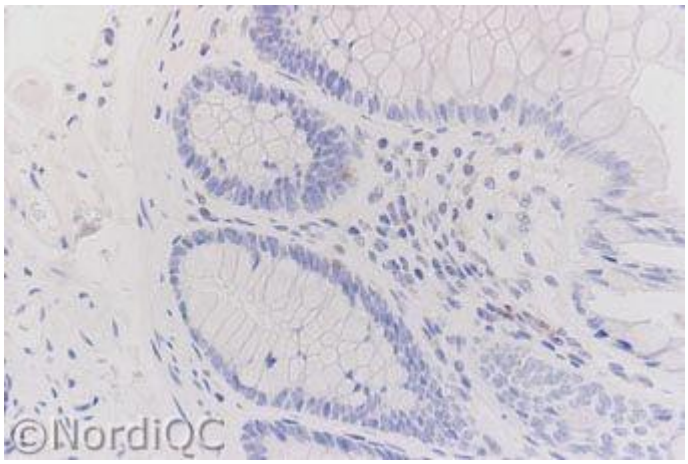

Fig. 2b
Insufficient CD3 staining of the colon using same protocol as in Fig. 1b. The T-cells are only weakly positive. Also compare with Fig. 3b.

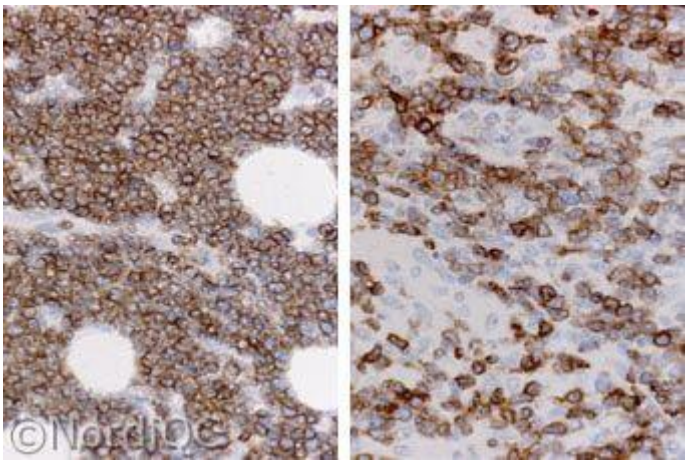

Fig. 3a
Optimal CD3 staining (left: Precursor T-cell lymphoma, right: Peripheral T-cell lymphoma)
using same protocol as used in Fig. 1a & 2a. Virtually all the neoplastic cells show a distinct staining and no background reaction.

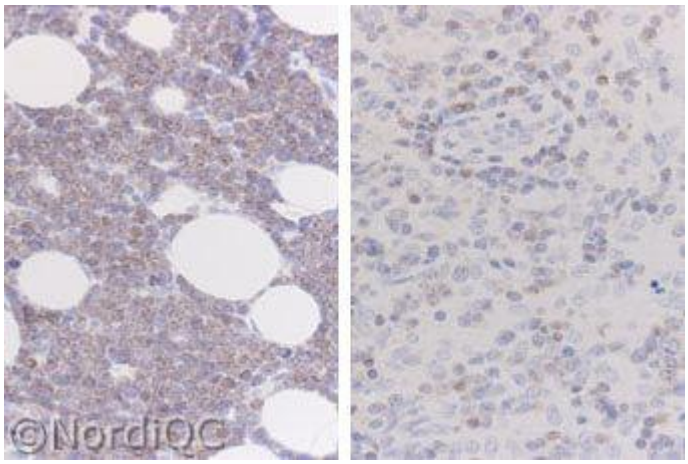

Fig. 3b
Insufficient CD3 staining (left: Precursor T-cell lymphoma, right: Peripheral T-cell lymphoma) using same protocol as used in Fig. 1b & 2b. The neoplastic cells only show a weak, diffuse staining.

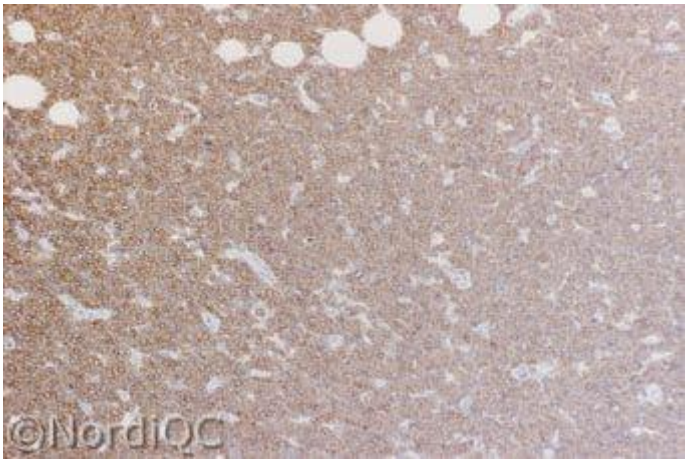

Fig. 4a
Uneven CD3 staining of the Precursor T-cell lymphoma using a protocol giving an otherwise optimal staining. The uneven reaction is most likely due to chromogen depletion that can be seen in antigen rich tissue. Increasing the volume of chromogen can reduce this problem.

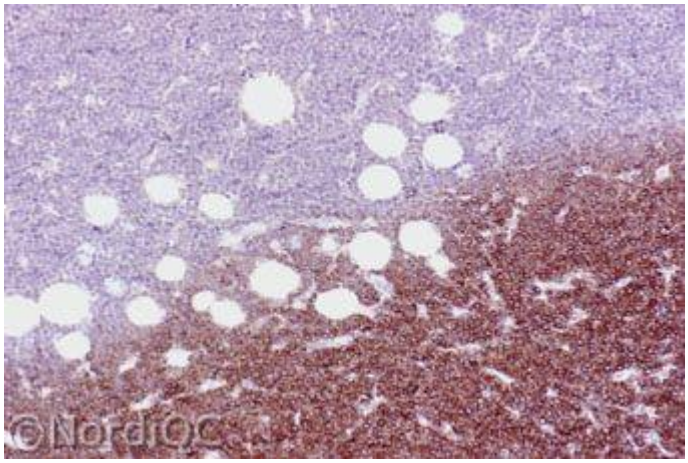

Fig. 4b
Uneven CD3 staining of the Precursor T-cell lymphoma. The upper part showing false negativity with a protocol giving an otherwise optimal staining. This sharp edge artefact is probably caused by a partial drying of the slides immediately after HIER.

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