# Assessment Run 37 2013
# CD3

**Material**
The slide to be stained for CD3 comprised:


1. Colon, 2. Tonsil, 3-4. Peripheral T-cell lymphoma, not-otherwise-specified (NOS)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD3 staining as optimal included:

- A moderate to strong and distinct predominantly membranous staining reaction of all T-cells both in the interfollicular T-zones and in the germinal centres of the tonsil.
- A moderate to strong and distinct predominantly membranous staining reaction of the intra-epithelial T-cells in the colon mucosa.
- An at least weak to moderate and distinct predominantly membranous staining reaction of the majority of the neoplastic T-cells in the two T-cell lymphomas.
- No staining of other cells. Especially the B-cells in the tonsil should be negative.

219 laboratories participated in the assessment. Of these 201 (92 %) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

Table 1. **Antibodies and assessment marks for CD3, run 37**

| Concentrated Antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **F7.2.38** | 24 | Dako | 16 | 2 | 6 | 0 | 75 % | 95 % |
| mAb clone **LN10** | 12 | Leica/Novocastra | 5 | 5 | 2 | 0 | 83 % | 100 % |
| mAb **PS1** | 25<br>3<br>2<br>1<br>1 | Leica/Novocastra<br>Monosan<br>Biocare<br>Gene Tech<br>Vector | 18 | 10 | 4 | 0 | 88 % | 92 % |
| rmAb **EP41** | 1 | Epitomics | 0 | 1 | 0 | 0 | - | - |
| rmAb **EP449E** | 1 | Epitomics | 1 | 0 | 0 | 0 | - | - |
| rmAb **SP7** | 18<br>1<br>1 | Thermo/NeoMarkers<br>Cell Marque<br>Zytomed | 6 | 11 | 3 | 0 | 85 % | 89 % |
| pAb **A0542** | 29 | Dako | 14 | 13 | 2 | 0 | 93 % | 96 % |
| Ready-To-Use Antibodies | | | | | | | | |
| mAb clone **LN10** **PA0553** | 10 | Leica/Novocastra | 10 | 0 | 0 | 0 | 100 % | 100 % |
| mAb clone **PS1** **CD3-PS1-R-7** | 1 | Leica/Novocastra | 0 | 1 | 0 | 0 | - | - |
| mAb clone **PS1** **PM110** | 1 | Biocare | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **2GV6** **790-4341** | 54 | Ventana | 51 | 3 | 0 | 0 | 100 % | 100 % |
| rmAb **EP272** **MAD-000325QD** | 1 | Master Diagnostica | 1 | 0 | 0 | 0 | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| rmAb clone **MRQ-39 103R** | 1 | Cell Marque | 1 | 0 | 0 | 0 | | |
| pAb **IR503/IS503** | 31 | Dako | 20 | 10 | 1 | 0 | 97 % | 97 % |
| pAb clone **N1580** | 1 | Dako | 0 | 1 | 0 | 0 | - | - |
| Total | 219 | | 144 | 57 | 18 | 0 | - | |
| Proportion | | | 66 % | 26 % | 8 % | 0 % | 92 % | |

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

**Detailed analysis of CD3, Run 37**
The following protocol parameters were central to obtain an optimal staining:

**Concentrated Antibodies**
mAb clone **F7.2.38**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9;Dako) (7/8) *, TRS pH 9 (Dako) (4/4), Borg Decloaker pH 9.5 (Biocare) (1/1), Tris-EDTA/EGTA pH 9 (3/6) or EDTA/EDTA pH 8 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 18 of 19 (95 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **LN10**: Protocols with optimal results were all based on HIER using either Bond Epitope Retrieval Solution 2 (BERS2; Leica) (2/4), BERS 1 (Leica) (1/2), Cell Conditioning 1 (CC1; Ventana) (1/2) or Citrate pH 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:140 depending on the total sensitivity of the protocol employed. Using these protocol settings 7 of 7 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **PS1**: Protocols with optimal results were all based on HIER using either TRS pH 9, 3-in-1 (Dako) (4/5), TRS pH 9 (Dako) (1/3), BERS 2 (Leica) (4/8), BERS 1 (Leica) (2/2), CC1 (Ventana) (5/10) or Tris-EDTA/EGTA pH 9 (2/3) as retrieval buffer. The mAb was typically diluted in the range of 1:40-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 24 of 26 (92 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **EP449E**: The protocol with an optimal result was based on HIER using CC1 (Ventana) (1/1) as retrieval buffer. The rmAb was diluted 1:250.

rmAb clone **SP7**: Protocols with optimal results were all based on HIER using either TRS pH 9, 3-in-1 (Dako) (1/1), TRS pH 9 (Dako) (1/1) or CC1 (Ventana) (4/11) as retrieval buffer. The rmAb was typically diluted in the range of 1:100-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 8 of 9 (89 %) laboratories produced a sufficient staining (optimal or good).

pAb **A0542**: Protocols with optimal results were all based on HIER using either TRS pH 9, 3-in-1 (Dako) (5/8), TRS pH 9 (Dako) (4/6), BERS 2 (Leica) (1/1), CC1 (Ventana) (2/11) or Tris-EDTA/EGTA pH 9 (2/3) as retrieval buffer. The pAb was typically diluted in the range of 1:50-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 23 of 24 (96 %) laboratories produced a sufficient staining (optimal or good).

Table 2 summarizes the overall proportion of optimal staining results when using the three most frequently used concentrated Abs on the three most commonly used IHC stainer platforms.

Table 2. **Optimal results for CD3 using concentrated antibodies on the 3 main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link / Classic** | | **Ventana BenchMark XT / Ultra** | | **Leica Bond III / Max** | |
|---|---|---|---|---|---|---|
| Buffer | TRS pH 9.0 | TRS pH 6.1 | CC1 pH 8.5 | CC2 pH 6.0 | ER2 pH 9.0 | ER1 pH 6.0 |
| mAb clone **F7.2.38** | 92 %<br>11/12** | - | 0 %<br>0/4 | 0 %<br>0/1 | - | - |
| mAb clone **PS1** | 63 %<br>5/8 | - | 50 %<br>5/10 | - | 50 %<br>4/8 | 100 %<br>2/2 |
| pAb **A0542** | 64 %<br>9/14 | - | 18 %<br>2/11 | - | 100 %<br>1/1 | - |

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

**Ready-To-Use (RTU) Antibodies**
mAb clone **LN10** (product. no. PA0553, Leica/Novocastra): Protocols with optimal results were all based on HIER using BERS 2, 15-30 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system.

mAb clone **PS1** (product. no. PM110, Biocare): The protocol giving an optimal result was based on HIER using Borg Decloaker pH 9.5 (Biocare) in a Pressure Cooker, 30 min incubation of the primary Ab and MACH4 (4U534) as detection system.

rmAb clone **2GV6** (prod. no. 790-4341, Ventana): Protocols with an optimal result were all based on HIER using mild or standard Cell Conditioning 1, 16-44 min incubation of the primary Ab and iView (760-091), UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 51 of 51 (100 %) laboratories produced an optimal staining.

pAb **IR503** (product.no. IR503, Dako): Protocols with an optimal result were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (heating time 10-20 min at 95-98°C), 20 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 28 out of 29 (97 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stainings were:

- Insufficient HIER (too short efficient HIER time)
- Too low concentration of the primary Ab
- Less successful performance of the mAb clone F7.2.38 on the BenchMark (Ventana) IHC platform

In this assessment and in concordance with the previous NordiQC assessments of CD3, the predominant reason for an insufficient result was a too weak staining reaction of the cells expected to be demonstrated. Virtually all laboratories could demonstrate CD3 in the T-cells in the interfollicular T-zones in the tonsils, whereas detection of CD3 in dispersed T-cells (e.g. in the mantle zones of germinal centres of the tonsil and in mucosa of the colon) was much more challenging and required an optimally calibrated protocol. If the dispersed T-cells only displayed a weak diffuse staining reaction, the neoplastic cells of the two peripheral T-cell lymphomas, NOS typically showed an equivocal staining reaction.

Several antibodies could be used to obtain an optimal staining result. In general, efficient HIER and a carefully calibrated Ab titre were the two main parameters for an optimal staining. No significant difference in the proportion of sufficient results was seen if either a 2-step or a more sensitive 3-step polymer/multimer system was used. Alkaline (pH 8-9) and acidic (pH 6) HIER buffers performed equally well.

In this assessment RTU systems from Dako, Leica and Ventana provided a slightly higher pass rate and proportion of optimal results, compared to in-house validated protocols for CD3.
The Leica and Ventana RTU systems, based on the mAb clones LN10 and 2GV6, gave the highest proportion of sufficient (both 100%) and optimal (100% and 94%, respectively) results. The Dako RTU system, based on a rabbit polyclonal antibody IR503/IS503, also provided a high pass (97 %), but with a lower proportion of optimal results (65 %). The lower proportion of optimal staining results for the Dako RTU system was primarily caused by a reduced signal-to-noise ratio and an aberrant cytoplasmic staining reaction of scattered epithelial cells in the colon mucosa. This pattern was also seen in the concentrated format of the Dako polyclonal Ab, A0542, and is most likely related to lot-to-lot variations for the the pAb. The pass rate of the mAb clone F7.2.38 seemed to be influenced by the IHC stainer used. When the clone was applied on the Dako Autostainer platform a pass rate of 92 % (n=11/12) was obtained, whereas all 5

protocols based on the same clone and similar protocol settings and applied on BenchMark platform gave an insufficient result (table 2).

**Controls**
Tonsil is recommendable control tissue for CD3 provided that the dispersed T-cells in the mantle zones and within the germinal centres display an at least moderate to strong and distinct membranous staining reaction. If these T-cells are only weakly stained, an inadequate staining in CD3 low-expressing T-cell lymphomas is seen (as observed in this and previous NordiQC assessments for CD3).
No staining must be seen in the germinal centre B-cells.

**Effect of external quality assessment**
This was the 3rd assessment of CD3 in NordiQC. A constant increase in the pass rate has been observed in these 3 runs as listed in table 3.

Table 3. **Proportion of sufficient CD3 staining results**

| | Run 14 2005 | Run 22 2008 | Run 37 2013 |
|---|---|---|---|
| Participants, n= | 87 | 119 | 219 |
| Sufficient results | 73 % | 84 % | 92 % |

The improvement is undoubtedly related to the high quality and extended use of RTU systems for CD3 from the three main providers, Dako, Leica and Ventana, with an overall pass rate of 99 %, superior to in-house validated assays.

**Conclusion**
The mAb clones **F7.2.38**, **LN10**, **PS1**, the rmAb clones **2GV6**, **EP449E**, **SP7** and the pAb **A0452** can all be recommended for demonstration of CD3.
Both concentrated formats and especially the Ready-To-Use systems from Dako, Leica and Ventana gave a high proportion of sufficient and optimal results. HIER is mandatory for a sufficient result.
Tonsil is an appropriate control for CD3. Both T-cells in the interfollicular areas and dispersed T-cells in the mantle zone and within the germinal centres must show a moderate to strong distinct membranous staining reaction, while B-cells must be negative.

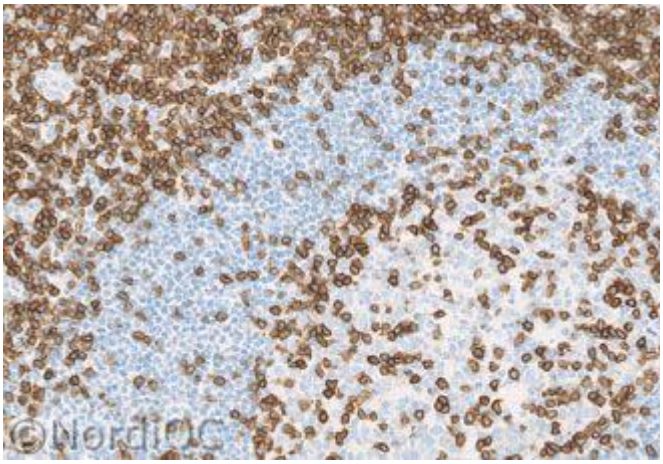



Fig 1a
Optimal CD3 staining of the tonsil using the rmAb clone 2GV6, Ready-To-Use, Ventana. Virtually all the T-lymphocytes in the T-zone and within the germinal centre show a strong and distinct membranous staining reaction. No background staining or staining of the B-cells is seen. Also compare with Figs. 2a – 3a, same protocol.

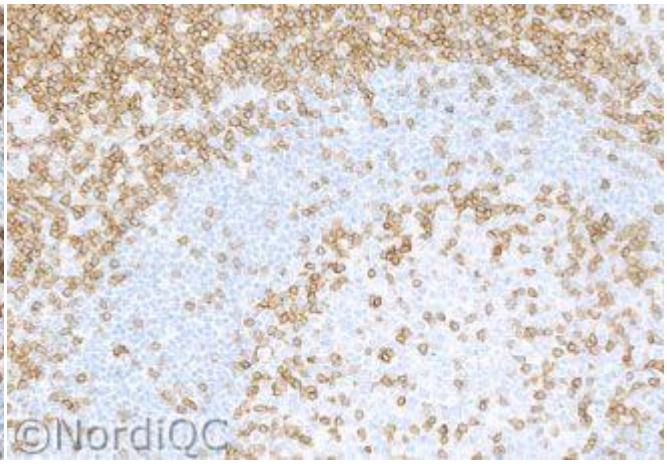



Fig 1b
Staining for CD3 of the tonsil using the mAb clone F7.2.38 by protocol settings giving a too low sensitivity - same field as in Fig. 1a.
The vast majority of the T-lymphocytes are demonstrated. A slightly weaker and less intense staining reaction is seen. However also compare with Figs. 2b – 3b, same protocol.

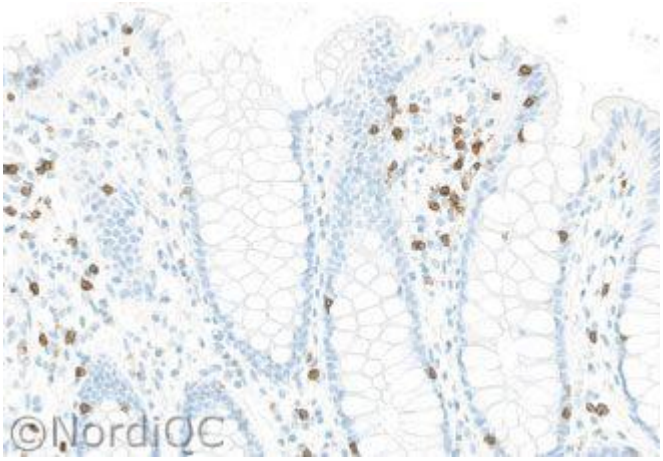



Fig 2a
Optimal CD3 staining of the colon using same protocol as in Fig. 1a.
The dispersed intraepithelial T-lymphocytes show a distinct staining reaction. The columnar epithelial cells are negative and no background staining is seen.

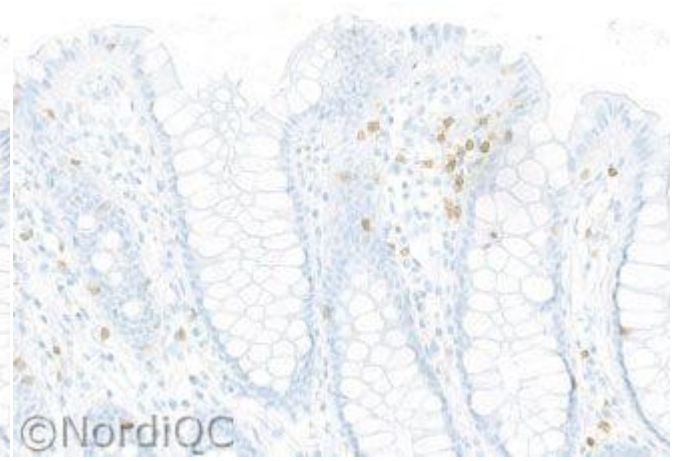



Fig 2b
Insufficient CD3 staining of the colon using same protocol as in Fig. 1b – same field as in Fig. 2a.
The intraepithelial T-lymphocytes are virtually negative.
Also compare with Fig. 3b, same protocol.

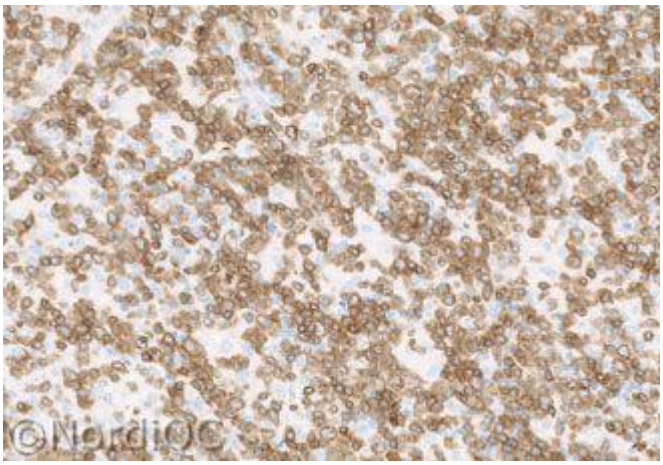



Fig 3a
Optimal CD3 staining of the peripheral T-cell lymphoma NOS using same protocol as in Figs. 1a & 2a.
Virtually all the neoplastic cells show a moderate to strong and distinct predominantly membranous staining reaction.
No background staining is seen.

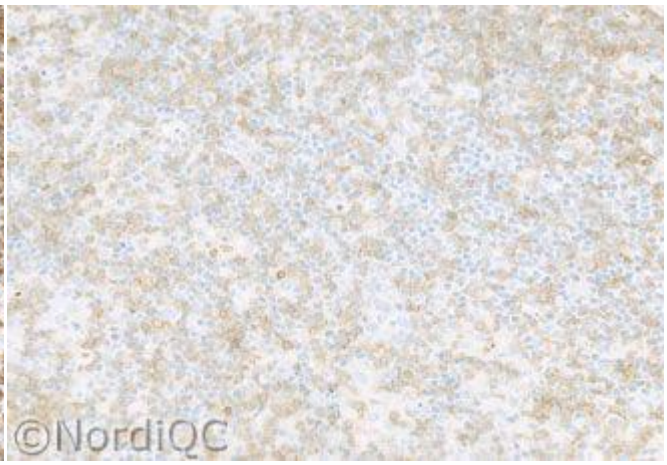
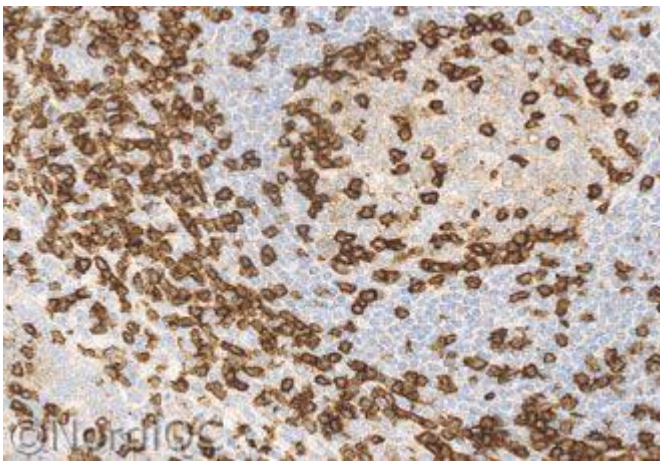



Fig 3b
Insufficient CD3 staining of the peripheral T-cell lymphoma NOS using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 3a.



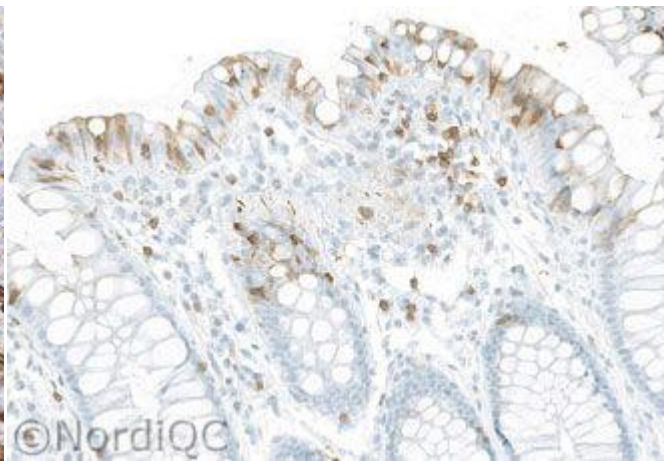



Fig 4a
Insufficient CD3 staining of the tonsil using the mAb clone PS1 too concentrated. The T-lymphocytes are demonstrated, but the germinal centre B-cells are false positive.

Fig 4b
Staining for CD3 of the colon using the pAb A0452, Dako. The T-lymphocytes are demonstratred, but an aberrant cytoplasmic staining is seen in the columnar epithelial cells. This staining pattern was frequently observed for protocols based on this antibody and might be related to lot-to-lot variations.

SN/RR/LE 25-2-2013