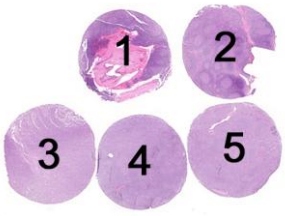
Assessment Run 34 2012
CD5

Material
The slide to be stained for CD5 comprised:



1. Tonsil, NBF 24h., 2. Tonsil, NBF 48h., 3. Mantle cell lymphoma (MCL), 4. & 5. B-CLL

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD5 staining as optimal included:

- A strong and distinct, predominantly membranous staining reaction of virtually all the T-cells in both the T-zones and within the germinal centres in the tonsils.
- An at least weak to moderate but distinct membranous staining reaction of dispersed B-cells in the mantle zone of the secondary follicles in the tonsils.
- An at least moderate and distinct membranous staining reaction of the majority of the neoplastic cells in the MCL and at least a weak to moderate staining reaction of the majority of the neoplastic cells of the two B-CLLs.
- No staining of the germinal centre B-cells.

A moderate to strong staining reaction in scattered squamous epithelial cells was accepted as this was seen for all the Abs used.

187 laboratories participated in this assessment. 79 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD5, run 34**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4C7**	62 9 6 5 2	Leica/Novocastra Dako Thermo/NeoMarkers Monosan Biocare	35	31	16	2	79 %	83 %
mAb clone **CD5/54/F6**	5	Dako*	0	0	1	4	0 %	-
rmAb clone **A25-G**	1	Master Diagnostica	0	0	1	0	-	-
rmAb clone **EP77**	1	Epitomics	0	1	0	0	-	-
rmAb clone **RBT-CD5**	1	Bio SB	0	1	0	0	-	-
rmAb **SP19**	14 3 2 1 1 1	Thermo/NeoMarkers Spring Bioscience Dako* Cell Marque Zeta Corporation Zytomed	7	9	5	1	73 %	77 %
pAb **E2474**	1	Spring Bioscience	0	1	0	0	-	-
Ready-To-Use Abs:								
mAb clone **4C7 IS/IR082**	14	Dako	6	6	2	0	86 %	90 %
mAb clone **4C7 PA0168**	6	Leica	4	2	0	0	100 %	100 %
mAb clone **4C7 PM099**	1	Biocare	0	1	0	0	-	-
mAb clone **4C7 CD5-4C7-R-7**	2	Novocastra	0	0	2	0	-	-

mAb clone **4C7 MS-393-R7**	1	Thermo/NeoMarkers	0	1	0	0	-	-
rmAb clone **SP19 790-4451**	33	Ventana	26	6	1	0	97 %	97 %
rmAb clone **SP19 IS/IR081**	10	Dako*	7	1	1	1	80 %	100 %
rmAb clone **SP19 760-4280**	3	Ventana/Cell Marque*	1	1	1	0	-	-
rmAb clone **SP19 205R-17**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **SP19 RMA-0593**	1	Maixin	0	0	0	1	-	-
Total	187		87	61	30	9	-	
Proportion			46 %	33 %	16 %	5 %	79 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below
* Product has been discontinued by the vendor

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

Concentrated Abs
mAb clone **4C7**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9; Dako) (11/18)*, TRS pH 9 (Dako) (2/6), Cell Conditioning 1 (CC1; BenchMark, Ventana) (4/18), Cell Conditioning 2 (CC2; BenchMark, Ventana) (1/1), Bond Epitope Retrieval Solution 2 (BERS 2; Bond, Leica) (7/14), Bond Epitope Retrieval Solution 1 (BERS 1; Bond, Leica) (2/2), Tris-EDTA/EGTA pH 9 (6/13), EDTA/EGTA pH 8 (1/3) or Citrate pH 6 (1/3) as the 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 53 out of 64 (83 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

rmAb clone **SP19**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either TRS pH 9 (3-in-1) (Dako) (1/2) or CC1 (BenchMark, Ventana) (6/12) as the retrieval buffer.
The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 10 out of 13 (77 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
mAb clone **4C7** (prod. no. IS/IR082, Dako): The protocols giving an optimal result were all based on HIER in PT-Link (heating time for 20 min at 97°- 98°C) using TRS pH 9 (3-in-1) as the HIER buffer, an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) as the detection system. Using these protocol settings 9 out of 10 (90 %) laboratories produced a sufficient staining.

mAb clone **4C7** (product.no. PA0168, Leica/Novocastra): The protocols giving an optimal result were all based on HIER using BERS 2 (Bond, Leica), an incubation time of 15-20 min in the primary Ab and Bond Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings 6 out of 6 laboratories (100 %) produced a sufficient staining.

rmAb clone **SP19** (prod. no. 790-4451, Ventana): The protocols giving an optimal result were based on HIER in CC1 (BenchMark, Ventana) mild or standard, an incubation time of 16-60 min in the primary Ab and iView (760-091) UltraView (760-500) with or without amplification kit or OptiView as the detection system.
Using these protocol settings 29 out of 30 (97 %) laboratories produced a sufficient staining.

rmAb clone **SP19** (prod. no. 205R-17, Cell Marque): The protocol giving an optimal result was based on HIER in CC1 (BenchMark, Ventana) standard, an incubation time of 32 min in the primary Ab and UltraView (760-500) as the detection system.

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody
- Insufficient HIER (too short efficient heating time)
- Use of detection systems with a too low sensitivity
- Less successful primary Ab

In this assessment and in concordance with the previous NordiQC assessments for CD5 the prevalent feature of an insufficient staining was a too weak or false negative staining reaction of the cells expected to be demonstrated. A too weak or false negative staining reaction was seen in 95 % of the insufficient results (37 out of 39) and in the remaining 5 % a poor signal-to-noise ratio was seen.
The majority of the laboratories could demonstrate CD5 in the normal T-cells in the two tonsils and in the infiltrating T-cells in the three small cell B-cell lymphomas, whereas the demonstration of CD5 in the neoplastic cells of both the two B-CLLs and the mantle cell lymphoma was much more challenging and required a correctly calibrated protocol.
A too low concentration of the primary Ab, insufficient HIER (too short efficient HIER time or HIER in a non-alkaline buffer) and/or the use of detection systems with a low sensitivity were the main reasons for the insufficient results of the mAb clone 4C7 and the rmAb clone SP19 when used as a concentrate.
It was observed that the pass rate and proportion of optimal results in particular was influenced by the sensitivity of the detection system used. If a 2-step polymer or multimer based detection system as e.g., EnVision Flex, Dako or UltraView, Ventana was used, 51 out of 76 laboratories obtained a sufficient staining result (67%) out of which 18 (24%) were assessed as optimal. If a more sensitive 3-step polymer or multimer based detection system as e.g., EnVision Flex+, Bond Refine (Leica) or UltraView + amplification was used, 33 out of 37 laboratories produced a sufficient staining result (89%) of which 22 (60%) were assessed as optimal.

All 5 protocols based on the mAb clone CD5/54/F6 were assessed as insufficient irrespective of the protocol settings being identical to the settings giving an optimal staining performance for both the mAb clone 4C7 and the rmAb clone SP19. The mAb clone CD5/54/F6 typically gave a satisfactory staining result in the normal T-cells with a high antigen expression, but gave a too weak or completely false negative staining reaction in cells with a reduced CD5 expression as both the normal mantle zone B-cells and the neoplastic cells of the mantle cell lymphoma and the B-CLLs. In total 33 protocols/slides based on the mAb clone CD5/54/F6 have been submitted to NordiQC in the last 3 runs for CD5 (runs 17, 24 and 34) and only 3 % (1 protocol) has provided a sufficient staining result (assessed as good) in these assessments. These data clearly supports that, this mAb clone can not be recommended for the demonstration of CD5 for diagnostic use and should be replaced in the laboratories. This has also been effectuated by Dako having discontinued the product and replaced this by the mAb clone 4C7.

For both the mAb clone 4C7 and the rmAb clone SP19 the Ready-To-Use (RTU) systems from Leica, Dako and Ventana, respectively, the obtained pass rates were higher than the pass rates obtained for the same clones used with an in-house validated assay based on the concentrated formats. However it has to be stressed that many different protocol settings for the RTU systems were applied by the laboratories and for some systems virtually none followed the recommended instructions given by the vendors of these systems. E.g. when focusing on the 26 protocols being based on the RTU system and the rmAb clone SP19 prod. no. 790-4451, Ventana giving an optimal staining result, all 26 protocols were based on a modified protocol compared to the recommended protocol given by Ventana. The modifications were typically based on a longer incubation time in the primary Ab (n=25 out of 26 optimal protocols) and/or a more sensitive detection system as the use of amplification kit or use of OptiView (n=13 out of 26 optimal protocols).

The tonsil was found to be a reliable control for CD5 provided that dispersed mantle zone B-cells showed an at least weak to moderate but distinct membranous staining reaction. If these cells were negative or only showed an equivocal and patchy membranous staining reaction, the sensitivity of the protocol was too low giving a false negative staining in the three B-cell lymphomas with a reduced CD5 expression. Virtual all T-cells in the T-zones and within the germinal centres must show a strong staining reaction, while the germinal centre B-cells must be negative. Scattered squamous epithelial cells, especially in lymphocyte infiltrating areas, showed a moderate to strong staining reaction.

This was the 4th assessment of CD5 in NordiQC (Table 2), and in this run a higher pass rate has been achieved compared to the level seen in the previous 3 runs, despite many new laboratories participated for the first time.

Table 2. **Proportion of sufficient results for CD5 in the four NordiQC runs performed**

	Run 8 2003	Run 17 2006	Run 24 2008	Run 34 2012
Participants, n=	65	88	119	187
Sufficient results	65 %	66 %	68 %	79 %

The significant improvement of the pass rate for CD5 was highly influenced by the reduced use of the less successful mAb clone CD5/54/F6 which only was used by < 3 % of the participants in this run compared to approximately 12 % in the previous run 24.
In this assessment for CD5 many new laboratories participated for the first time and for these a slightly lower pass rate was observed compared to the laboratories also participating in the previous run 24, 2008: For the laboratories participating for the first time the pass rate was 74 % (62 out of 84), whereas the pass rate was 83 % (86 out of 103 laboratories) for the laboratories participating in both runs.

Conclusion
The mAb clone 4C7 and the rmAb clone SP19 are both recommendable Abs for the demonstration of CD5. Efficient HIER in an alkaline buffer in combination with a sensitive detection system was mandatory for an optimal performance. The protocol must be carefully calibrated in order to detect the low expression of CD5 in the small cell B-cell lymphomas as B-CLL and mantle cell lymphoma.
Normal tonsil is an appropriate control provided that at least a weak to moderate but distinct membranous staining reaction is seen in dispersed B-cells in the mantle zone of the secondary follicles in the tonsils. No staining must be seen in the germinal centre B-cells.

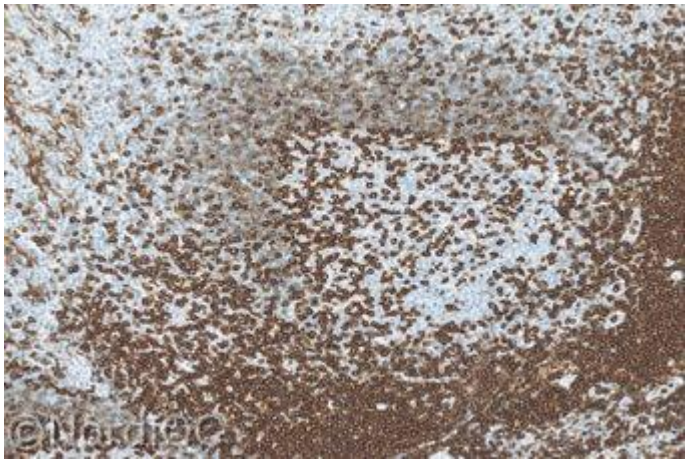


Fig. 1a
Optimal staining for CD5 of the tonsil no. 1 fixed 24 h. in NBF using the rmAb clone SP19 optimally calibrated, HIER in an alkaline buffer and a 3-step multimer conjugate.
The T-cells in the interfollicular T-zone and within the germinal centre show a strong staining reaction. Dispersed B-cells show a moderate staining reaction, which even at low magnification (x100) can be identified.
Also compare with Figs. 2a – 4a, same protocol.

Fig. 1b
Insufficient staining for CD5 of the tonsil no. 1 using the mAb clone 4C7 by a protocol with a too low sensitivity (too low. conc. of the primary Ab and a 2-step polymer conjugate) - same field as in Fig. 1a.
The intensity of the staining reaction in the T-cells is reduced compared to the result obtained in Fig. 1a, but most critical is the negative staining reaction in the mantle zone B-cells.
Also compare with Figs. 2b - 4b, same protocol.

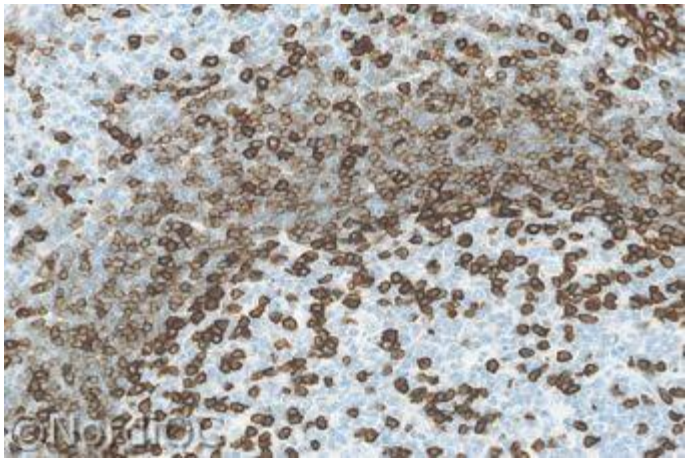


Fig. 2a
Optimal staining for CD5 of the tonsil no. 1 using same protocol as in Fig. 1a – high magnification x200.
Dispersed mantle zone B-cells show a distinct moderate staining reaction, while the T-cells show a strong staining reaction. No staining is seen in the germinal centre B-cells.

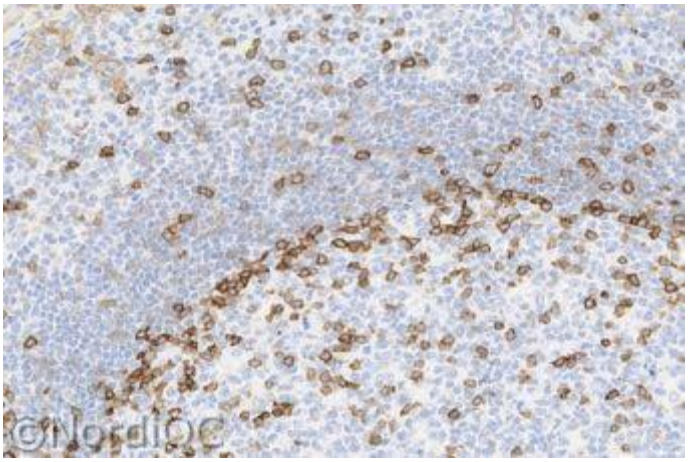


Fig. 2b
Insufficient staining for CD5 of the tonsil no. 1 using same protocol as in Fig. 1b - same field as in Fig. 2a – high magnification x200
Only the T-cells show a distinct staining reaction, while the mantle zone B-cells are false negative.

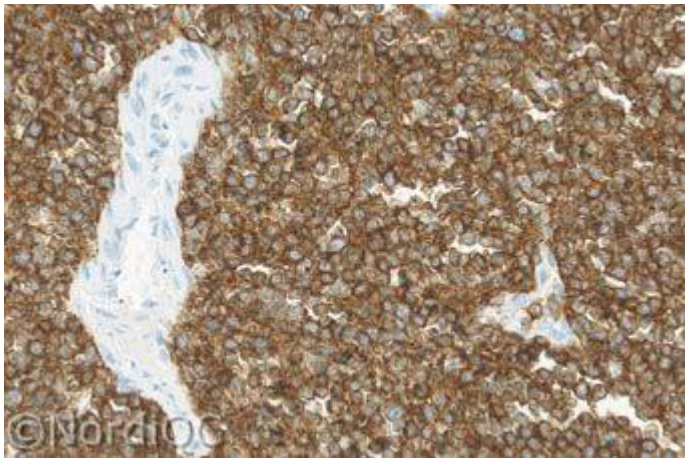


Fig. 3a
Optimal staining for CD5 of the mantle lymphoma using same protocol as in Figs. 1a - 2a. Virtually all the neoplastic cells show a moderate to strong distinct staining reaction. No background staining is seen.

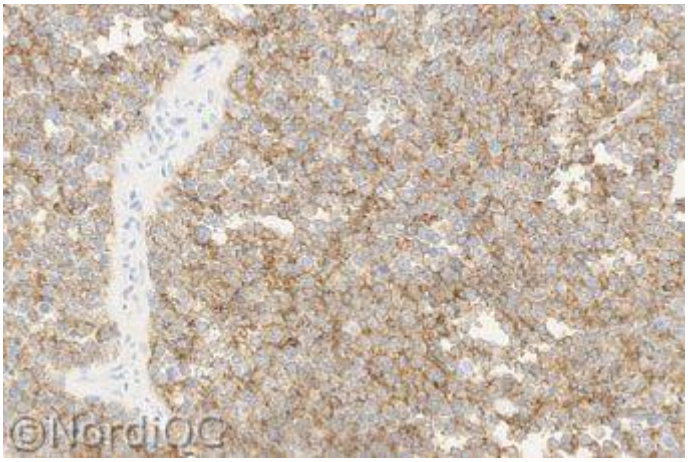


Fig. 3b
Staining for CD5 of the mantle cell lymphoma using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
The proportion and the intensity of the neoplastic cells are significantly reduced compared to the result obtained in Fig. 3a. However also compare with Fig. 4b.

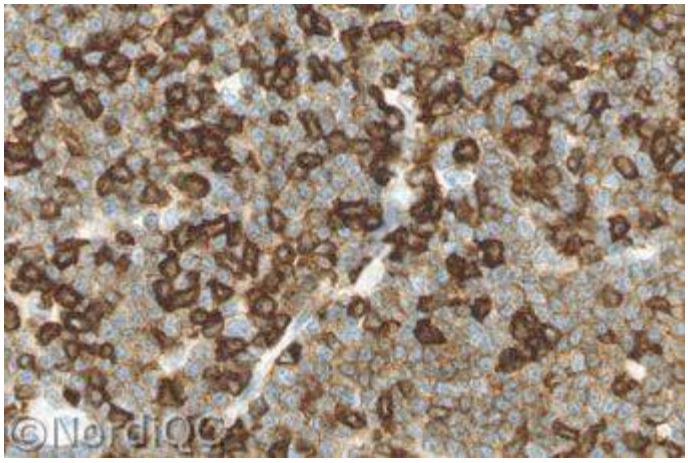


Fig. 4a
Optimal staining for CD5 of the B-CLL no. 5 using same protocol as in Figs. 1a - 4a.
The majority of the neoplastic cells show a moderate and distinct staining reaction, while the infiltrating normal T-cells show a strong staining reaction.

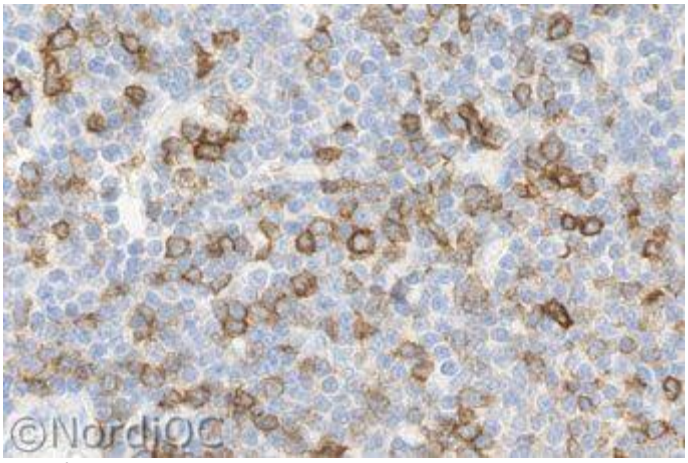


Fig. 4b
Insufficient staining for CD5 of the B-CLL using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The neoplastic cells are virtually negative and only the normal T-cells are clearly demonstrated.

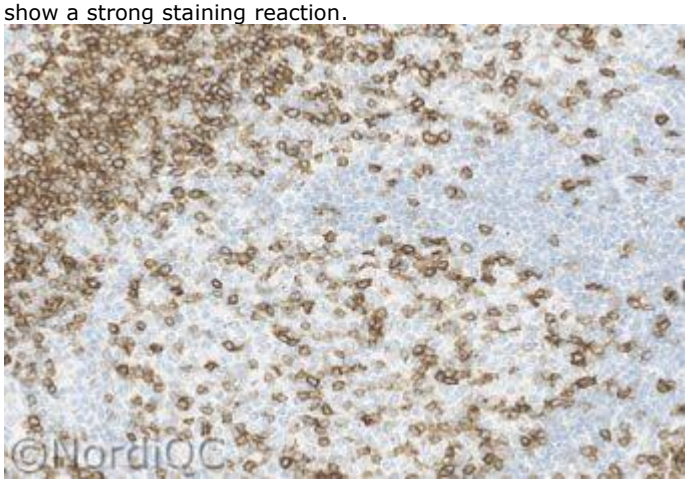


Fig. 5a
Insufficient staining for CD5 of the tonsil no. 1 using the mAb clone CD5/54/F6.
Despite a high titre of the primary Ab, efficient HIER in an alkaline buffer and a 3-step polymer conjugate is used, only the T-cells are demonstrated. No staining reaction is seen in the mantle zone B-cells. Also compare with Fig. 5b, same protocol.
This mAb clone has constantly shown this insufficient performance and should not be used for diagnostic purpose.

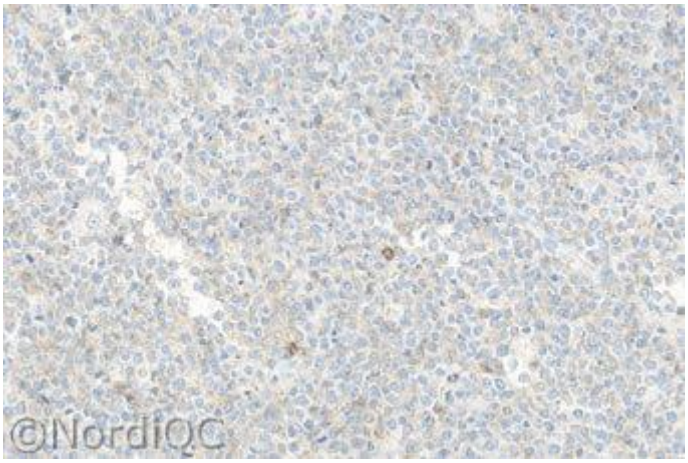


Fig. 5b
Insufficient staining for CD5 of the mantle cell lymphoma using same protocol as in Fig. 5a.
The neoplastic cells are virtually negative and only the normal T-cells are clearly demonstrated.

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