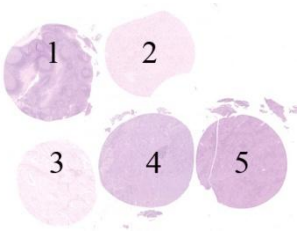
CD10

Assessment Run 39 2013

The slide to be stained for CD10 comprised:



1. Tonsil, 2. Kidney, 3. Renal clear cell carcinoma, 4. Burkitt lymphoma, 5. Follicular lymphoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CD10 staining as optimal were:

- An at least moderate, distinct membranous staining reaction of virtually all germinal centre B-cells in the tonsil.
- A moderate to strong, predominantly membranous but also cytoplasmic staining reaction of virtually all the epithelial cells in the renal proximal tubules and the parietal layer of the Bowman's capsule.
- An at least moderate staining reaction of virtually all neoplastic cells in the renal clear cell carcinoma and Burkitt lymphoma.
- An at least weak staining reaction of the vast majority of neoplastic cells of the follicular lymphoma.
- An at least weak to moderate staining of neutrophil granulocytes in all the specimens.

230 laboratories participated in this assessment. 209 (91 %) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in table 1 (page 2).

Detailed analysis of CD10, Run 39

The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies
mAb clone **56C6**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (11/16)*, TRS pH 9 (Dako) (5/9), Cell Conditioning 1 (CC1; Ventana) (36/53), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (19/21), Diva Decloaker pH 6.2 (Biocare) (1/1), Tris-EDTA/EGTA pH 9 (8/13) or EDTA/EGTA pH 8 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:10-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 104 of 110 (95 %) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
mAb clone **56C6**, product no. IS/IR648, Dako, Autostainer+/Autostainer Link: Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min at 95-98°C) and 20-30 min.incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 42 of 43 (98 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **56C6**, product no. GA648, Dako, Omnis: The protocol with optimal result was based on HIER in TRS pH 9 (3-in-1) (efficient heating time 30 min at 97°C) and 12.5 min. incubation of the primary Ab and EnVision FLEX+ (DM847) as detection system.

mAb clone **56C6**, product no. PA0270, Leica, Bond-max/Bond-III: Protocols with optimal results were all based on HIER using BERS 2 pH 9 (Bond, Leica) (efficient heating time 10-20 min. at 97-100°C), 15-30 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 7 of 7 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **56C6**, product no. PM129, Biocare, IntelliPath: The protocol with optimal result was based on HIER in a Pressure Cooker using Borg Decloaker pH 9.5 (efficient heating time 15 min. at 110°C), 30 min incubation of the primary Ab and MACH4 (M4U534) as detection system.

Table 1. **Antibodies and assessment marks for CD10, run 39**

Concentrated antibodies	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **56C6**	80 14 9 6 4 4 1 1 1 1	Leica/Novocastra Dako Thermo/NeoMarkers Monosan Biocare Cell Marque Diagnostic Biosystems DCS Nordic Biosite Vector	81	32	6	2	93 %	95 %
rmAb clone **EP195**	1	Diagnostic Biosystems	0	1	0	0	-	-
rmAb clone **G27-P**	1	Biotech	0	0	0	1	-	-
Ready-To-Use antibodies								
mAb clone **56C6 IS648/IR648**	47	Dako	36	10	1	0	98 %	98 %
mAb clone **56C6 GA648**	1	Dako	1	0	0	0	-	-
mAb clone **56C6 PA0270**	7	Leica	6	1	0	0	100 %	100 %
mAb clone **56C6 110M-18**	3	Cell Marque	2	1	0	0	-	-
mAb clone **56C6 PM129**	1	Biocare	1	0	0	0	-	-
mAb clones **56C6 PDM107**	1	Diagnostic Biosystems	1	0	0	0	-	-
mAb clone **56C6 GT200402**	1	Gene Tech	0	0	1	0	-	-
rmAb clone **56C6 CD10-270-R-7**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **56C6 MAD-002022QD**	1	Master Diagnostica	0	1	0	0	-	-
mAb clone **56C6 MSG070**	1	Zytomed	1	0	0	0	-	-
rmAb clone **SP67 790-4506**	43	Ventana	9	24	10	0	79 %	96 %
Total	230		138	71	18	3	-	
Proportion			60 %	31 %	8 %	1 %	91 %	

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

rmAb clone **SP67**, product no. 790-4506, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were all based on HIER using mild, standard or extended Cell Conditioning 1, 4-68 min. Incubation of the primary Ab and UltraView (760-500 + amplification kit) or OptiView (760-700 +/- amplification kit) as detection system. Using these protocol settings 23 of 24 (96 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient staining reactions were:

- Too low concentration of the primary antibody
- Use of low sensitive detection systems
- Inappropriate epitope retrieval (proteolysis)
- Use of non-alkaline HIER buffer or too short efficient HIER time

In this assessment and in concordance with the previous NordiQC assessments of CD10, the prevalent feature of insufficient staining was a too weak or false negative staining reaction. This was observed in all the 21 results evaluated as insufficient. In two of the insufficient cases a false positive staining due to endogenous biotin was also observed. Virtually all laboratories could demonstrate CD10 in the renal proximal tubules and the renal cell carcinoma whereas the germinal centre B-cells in tonsil and the neoplastic cells of the follicular lymphoma were more challenging and required an optimally calibrated protocol.

The mAb clone 56C6 was the most widely used antibody for CD10. Applied as a concentrate, high proportions of sufficient results were seen. Optimal staining results could be obtained on all main IHC platforms (Dako, Ventana

and Leica, see table 2). Efficient HIER in an alkaline buffer, a sensitive non-biotin based detection system and titre in the range of 1:10-100 were the main protocol prerequisites for an optimal result. Especially use of 3-step polymer / multimer based detection systems provided higher proportions of optimal results.

Table 2. **Optimal results for CD10 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	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 **56C6**	64 % 14/22**	0 % 0/1	67 % 35/52	-	95 % 19/20	0 % 0/1

* 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)

Protocols based on 2-step polymer/multimer detection systems, provided a sufficient staining result in 91% (n=42/46), and in 52% (n=24/46) an optimal result was obtained. If the same protocol settings were applied with a 3-step polymer/multimer based detection system (EnVision FLEX+ (Dako), Optiview (Ventana) or Bond Refine (Leica)), sufficient staining results were seen in 100% (n=58/58), and 86% (n=50/58) was evaluated as optimal. This is also reflected in the high proportion of optimal results (95 %) using the mAb clone 56C6 as concentrate on the Leica IHC platforms (table 2), on which a 3-step polymer based detection system is used as standard.

Corresponding RTU systems based on the mAb clone 56C6 (primarily from Dako and Leica) also provided a high proportion of sufficient and optimal results. The Ventana RTU system based on the rmAb clone SP67 gave a smaller proportion of sufficient results of 79 % (n=33/43) out of which 21 % (n=9/43) were evaluated as optimal. It was observed, that the officially recommended protocol for the Ventana RTU format prod. no. 790-4506 (extended HIER in CC1, 16-20 min. incubation of the primary Ab and UltraView as detection system) was less successful as no optimal results was obtained by these protocol settings. All 9 optimal results produced with the Ventana RTU system was based on modified and laboratory validated protocol settings typically based on OptiView or UltraView with amplification.

Controls
Tonsil is the recommended control for CD10. Virtually all the germinal centre B-cells must show an at least moderate but distinct membranous staining reaction. The mantle zone B-cells and squamous epithelial cells must be negative. Scattered neutrophils and endothelial cells will typically show a weak to strong staining reaction.

Performance history
This was the 4th NordiQC assessment of CD10. A significant increase in the pass rate was seen compared to previous runs.

Table 3: **Proportion of sufficient results for CD10 in the four NordiQC runs performed**

	Run 6 2002	Run 16 2006	Run 27 2009	Run 39 2013
Participants, n=	43	89	137	229
Sufficient results	63 %	72 %	74 %	91 %

Several parameters may contribute to the improved pass rate. Increased use of sensitive and specific polymer/multimer based detection systems in combination with a reduced use of non-alkaline buffers for HIER seem to be the main explanation. In run 6, 2002 biotin-based detection systems were used by 47 % of the participants and 23 % performed HIER by the use of citrate pH 6. These protocol settings resulted in low sensitivity and false positive staining reactions due to endogenous biotin. In this run 4 % of participants used a biotin-based detection system and 2 % HIER in a non-alkaline buffer as citrate pH 6.

Conclusion
The mAb clone **56C6** is a highly recommendable marker for CD10. Efficient HIER in alkaline buffer in combination with a sensitive detection system gave the highest proportion of optimal results. The concentrated format of the mAb clone **56C6** provided an optimal result on all the 3 main IHC platforms (Ventana, Dako and Leica). The corresponding RTU systems based on the mAb clone **56C6** from Dako and Leica also provided a high proportion of sufficient and optimal results. RTU rmAb clone **SP67** (Ventana) can only give an optimal result, if a more sensitive visualization system than recommended by the vendor is applied.
Tonsil is the recommended control for CD10: Virtually all the germinal centre B-cells must show an at least moderate and distinct membranous staining reaction. The mantle zone B-cells and squamous epithelial cells must be negative.

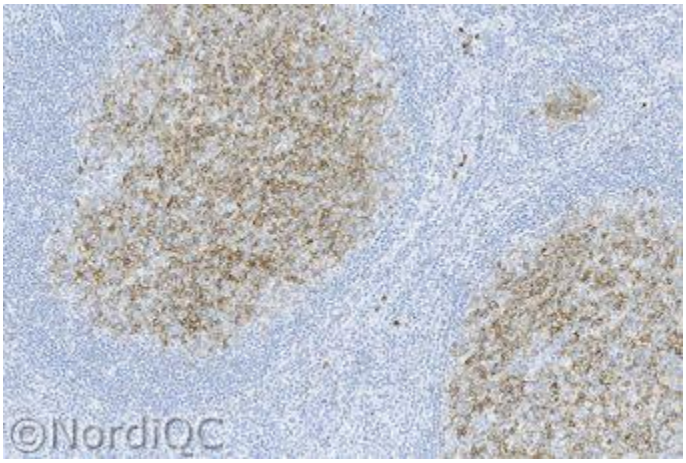


Fig. 1a
Optimal CD10 staining of the tonsil using the mAb clone 56C6 optimally calibrated and with HIER in an alkaline buffer. Virtually all the germinal centre B-cells show a strong and distinct membranous staining reaction. No staining of mature lymphocytes is seen. Also compare with Figs. 2a - 4a, same protocol.

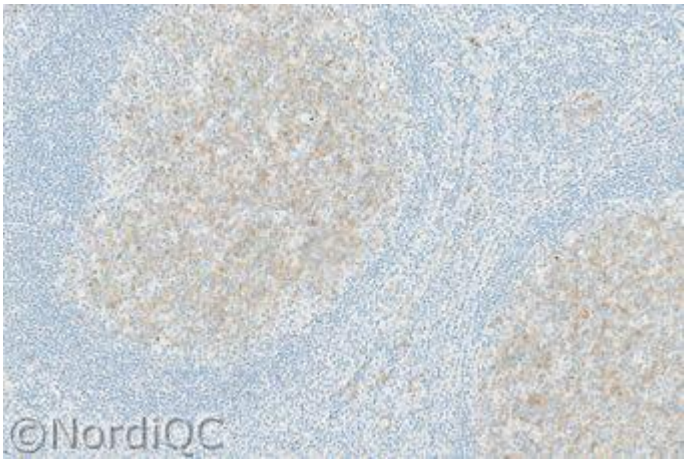


Fig. 1b
Insufficient CD10 staining of the tonsil using the mAb clone 56C6 with protocol settings giving a too low sensitivity - same field as in Fig. 1a. The germinal centre B-cells only show a weak and equivocal staining reaction. Also compare with Figs. 2b - 4b, same protocol.

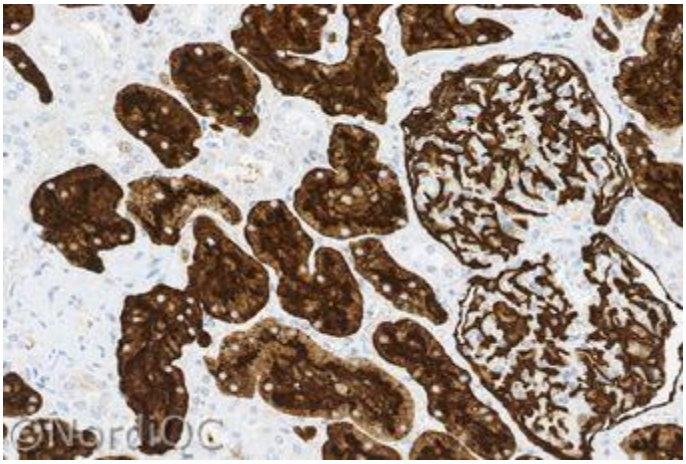


Fig. 2a
Optimal CD10 staining of the kidney using protocol as in Fig. 1a. The epithelial cells of the proximal tubules and glomeruli show an intense membranous and cytoplasmic staining reaction. No background staining is seen.

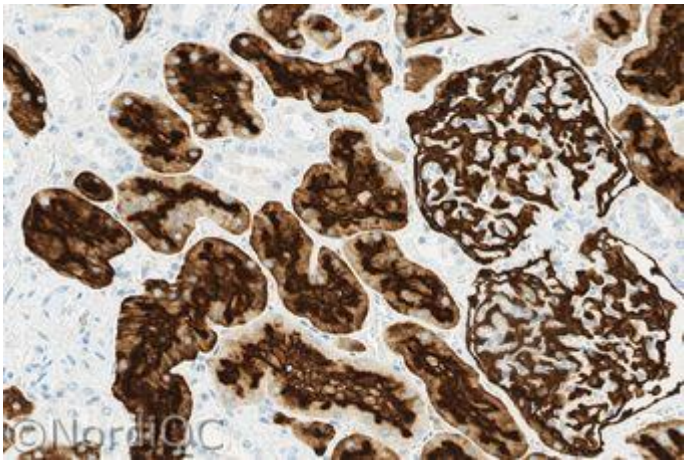


Fig. 2b
CD10 staining of the kidney using same insufficient protocol as in Fig. 1b - same field as in Fig. 2a. The epithelial cells of the proximal tubules and glomeruli show a strong membranous and cytoplasmic staining reaction. These structures have a high level of antigen expression and thus cannot be used to calibrate the protocol for CD10. See Figs. 1b, 3b & 4b – same protocol.

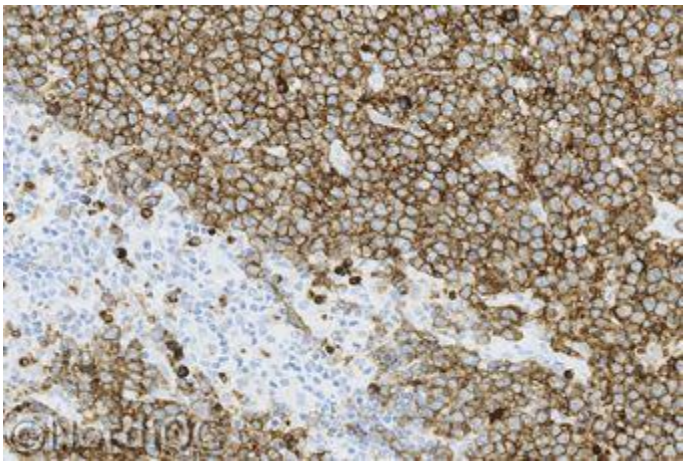


Fig. 3a
Optimal CD10 staining of the Burkitt lymphoma using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct membranous staining reaction.

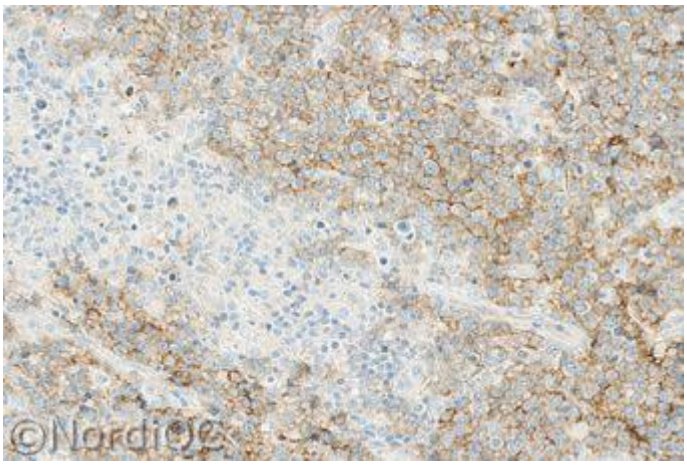


Fig. 3b
Insufficient CD10 staining of the Burkitt lymphoma using same protocol as in Figs. 1b & 2b - same field as in Fig. 3a. The neoplastic cells only show a weak, diffuse staining reaction.

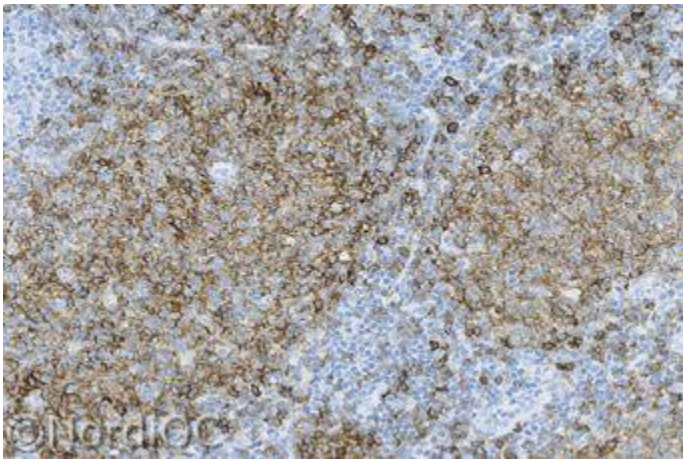


Fig. 4a.
Optimal CD10 staining of the follicular lymphoma using same protocol as in Figs. 1a - 3a. The majority of the neoplastic cells show a moderate to strong and distinct membranous staining reaction.

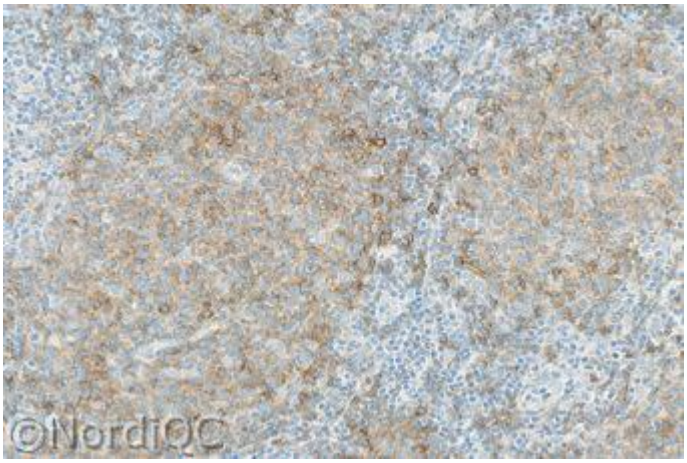


Fig. 4b.
Insufficient CD10 staining of the follicular lymphoma using same protocol as in Figs. 1b – 3b – same field as in Fig. 4a. The neoplastic cells only show a weak, diffuse staining reaction.

SN/RR/LE/MV 07-12-2013