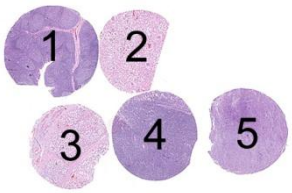
Assessment Run 27 2009
CD10

The slide to be stained for CD10 comprised:
1. Tonsil, 2. Kidney, 3. Renal clear cell carcinoma, 4. Burkitt lymphoma,
5. Endometrial stromal sarcoma.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a CD10 staining as optimal included:

- A moderate to strong distinct membranous staining of virtually all the germinal centre B-cells in the tonsil.
- A moderate to strong predominantly membranous but also cytoplasmic staining of virtually all the epithelial cells of the proximal tubules and the epithelial cells lining the Bowman capsule in the kidney.
- An at least moderate staining of virtually all the neoplastic cells in the the renal clear cell carcinoma and Burkitt lymphoma.
- An at least weak staining of scattered neoplastic cells of the endometrial stromal sarcoma.
- An at least weak to moderate staining of the neutrophilic granulocytes in all the specimens.

137 laboratories participated in this assessment. 74 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD10, run 27**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **56C6**	74 7 6 4 3 2 1 1	Novocastra Monosan NeoMarkers Biocare Dako Vector Cell Marque Master Diagnostica	40	33	17	8	74 %	79 %
Ready-To-Use Abs								
mAb clone **56C6, IS648/IR648**	14	Dako	8	6	0	0	100 %	100 %
mAb clone **56C6, 760-2705**	22 2	Ventana Cell Marque	4	11	5	4	63 %	94 %
mAb clone **56C6, IP129**	1	Biocare	0	0	1	0	-	-
Total	137		52	50	23	12	102	-
Proportion			38 %	36 %	17 %	9 %	74 %	-

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:

Concentrated Abs
mAb clone **56C6**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) in one of the following buffers: Tris-EDTA/EGTA pH 9 (12/32)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (13/23), Bond Epitope Retrieval Solution 2 (Bond, Leica) (9/13), Cell Conditioning 1 (BenchMark, Ventana) (4/15), EDTA/EGTA pH 8 (1/6) or Citrate pH 6 (1/7) 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 70 out of 89 (79 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Ready-To-Use Abs
mAb clone **56C6** (prod. no IS648/IR648, 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 or 30 min in

the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 14 (100 %) laboratories produced a sufficient staining.

mAb clone **56C6** (prod. no. 760-2705, Ventana/Cell Marque): The protocols giving an optimal result were based on HIER using standard Cell Conditioning 1 (BenchMark, Ventana), an incubation time of 32 – 120 min in the primary Ab and Ultra View (760-500) as the detection system. 2 labs used amplification kit. Using these protocol settings 15 out of 16 (94 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- Insufficient HIER
- Usage of a biotin based detection system (giving a false positive reaction).

In this assessment and in concordance with the results in the previous CD10 assessments, the prevalent feature of an insufficient staining was a too weak or false negative reaction. This was observed in all the 35 stains assessed as insufficient. In 5 of the insufficient cases (14%) also a false positive staining due to endogenous biotin was observed. The weak or false negative staining were characterized by a diffuse staining of the germinal centre B-cells in the tonsil and the neoplastic cells of the Burkitt lymphoma and a completely negative staining of the neoplastic cells of the endometrial sarcoma. Virtually all laboratories were able to demonstrate CD10 in the normal epithelial cells of the proximal tubules and the neoplastic cells of the renal cell carcinoma. Using a 3-step polymer or multimer based system (e.g., EnVision Flex+, Dako, or PowerVision+, ImmunoVision) 54 out of 60 laboratories (90%) obtained a sufficient result, of which 36 were optimal (60%).
An optimal result could not be obtained when a biotin based detection system was used, as the combination of efficient HIER and a biotin based detection system resulted in a false positive reaction of endogenous biotin in the renal tubules.

This was the third assessment of CD10. A slight increase in the overall pass rate has been achieved as shown in table 2, despite the large number of new participants.

Table 2. **Proportion of sufficient results for CD10 in the three NordiQC runs performed**

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

In the previous assessments of CD10 (run 6 and 16), a total of 41 laboratories obtaining an insufficient result was given specific recommendations how to improve their protocol – typically to increase the concentration of the primary Ab and/or to prolong HIER. 35 of these submitted a new stain in the subsequent run. 18 followed the recommendations, of which 16 improved to good or optimal (89%). 9 laboratories did not follow the recommendations, and 2 of these (22 %) obtained a sufficient staining in the subsequent run. 8 laboratories changed their IHC system completely and 6 of these obtained a sufficient result.

Conclusion
The mAb clone 56C6 is currently the only Ab applicable for CD10 on FFPE material. HIER, preferable in an alkaline buffer, is mandatory for an optimal result. The concentration of the primary Ab should be carefully calibrated. A 3-step polymer or multimer detection system seems to be favourable for an optimal performance. Tonsil is an appropriate control for CD10: Virtually all the germinal centre B-cells must show a moderate to strong distinct membranous staining.

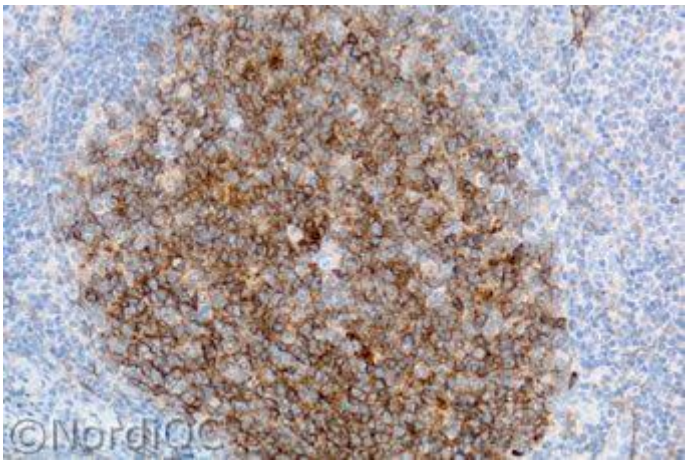


Fig. 1a
Optimal CD10 staining of the tonsil using the mAb clone 56C6 optimally calibrated after HIER in an alkaline buffer. Virtually all the germinal centre B-cells show a strong and distinct membranous staining without any staining of mature lymphocytes.

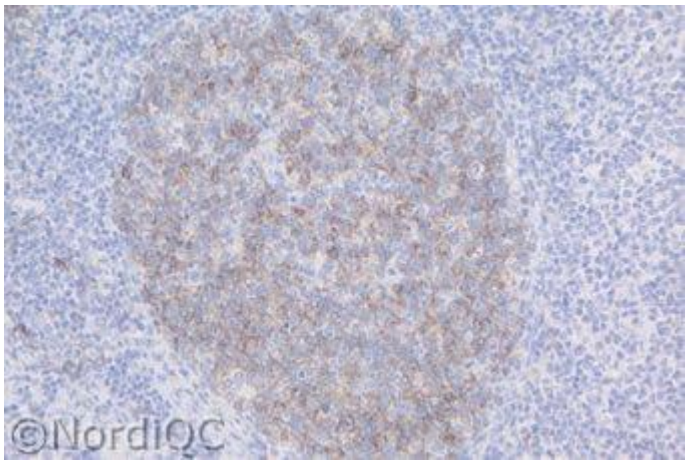


Fig. 1b
Insufficient CD10 staining of the tonsil using the mAb clone 56C6 too diluted - same field as in Fig. 1a. The germinal centre B-cells only show a weak and diffuse staining – also compare with Figs. 2b – 4b (same protocol).

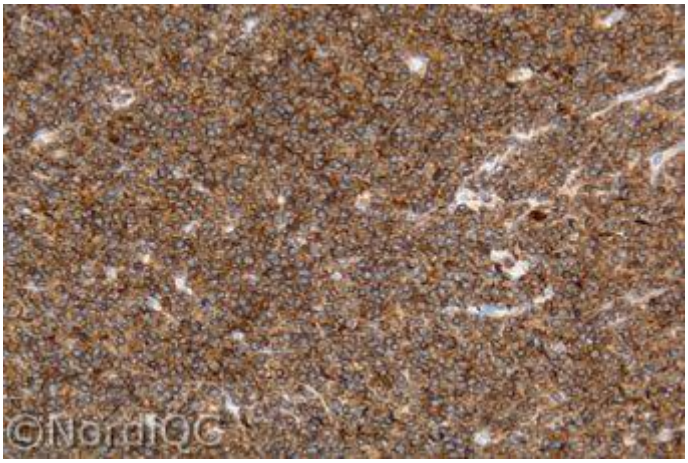


Fig. 2a
Optimal CD10 staining of the Burkitt lymphoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct staining.

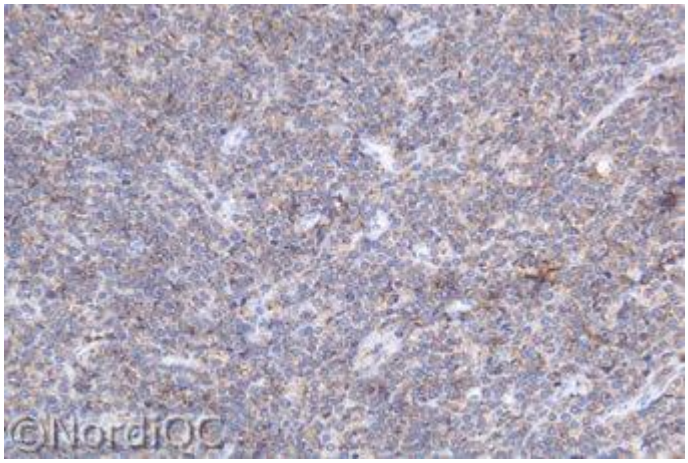


Fig. 2b
Insufficient CD10 staining of the Burkitt lymphoma using same protocol as in Fig. 1b – same field as in Fig 2a. The neoplastic cells only show a faint, equivocal staining.

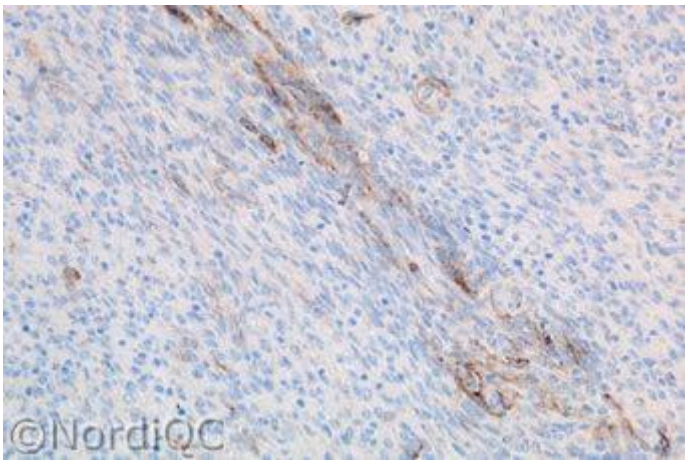


Fig. 3a
Optimal CD10 staining of the endometrial stromal sarcoma using same protocol as in Fig. 1a & 2a. Scattered neoplastic cells show a moderate, distinct staining.

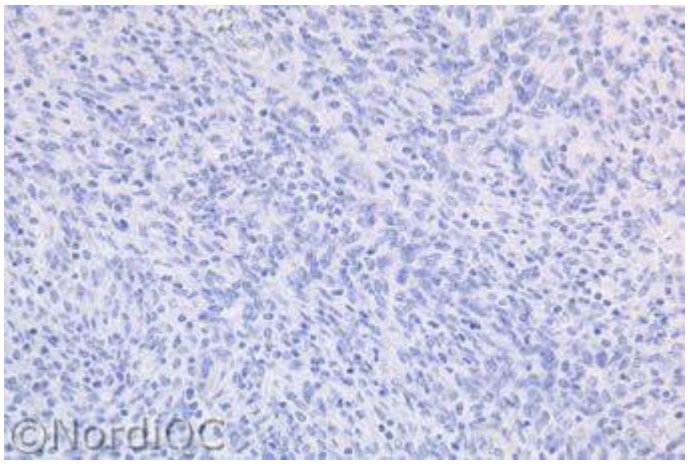


Fig. 3b
Insufficient CD10 staining of the endometrial stromal sarcoma using same protocol as in Fig. 1b & 2b – same field as in Fig. 3a. The neoplastic cells are false negative.

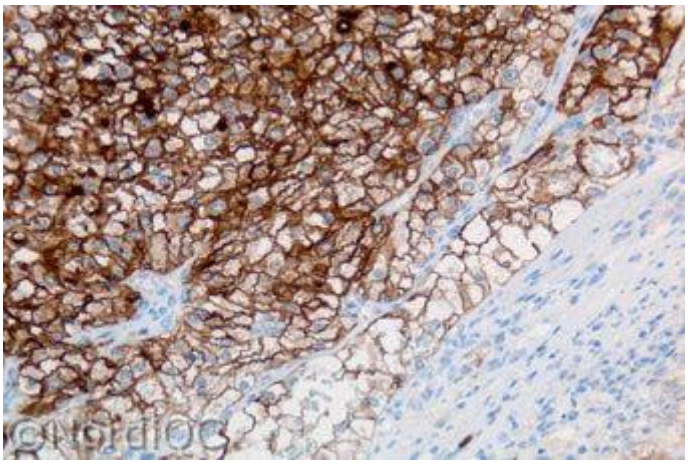


Fig. 4a
Optimal CD10 staining of the renal clear cell carcinoma using same protocol as in Fig. 1a – 3a. Virtually all the neoplastic cells show a strong and distinct staining.

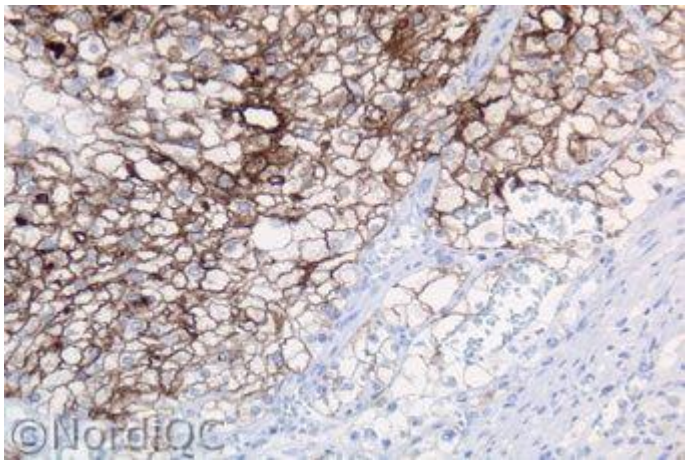


Fig. 4b
CD10 staining of the renal clear cell carcinoma using same insufficient protocol as in Fig. 1b – 3b, same field as in Fig. 4a. Almost all the neoplastic cells are labelled, indicating that these cells have a high antigen expression compared to the expression in, e.g., germinal centre cells and Burkitt lymphoma as illustrated in Figs. 1b & 2b.

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