Assessment Run 16 2006

CD10

The slide to be stained for CD10 comprised:
1. Tonsil, 2. Liver, 3. B-cell chronic lymphatic leukaemia (B-CLL), 4. Burkitt lymphoma, 5. Follicular lymphoma (high grade), 6. Clear cell renal carcinoma.
All specimens were fixed in 10 % NBF.

Criteria for assessing a CD10 staining as optimal included:

- A strong, distinct membranous staining reaction of the normal germinal centre B-cells in the tonsil.
- A strong, distinct staining reaction of bile canaliculi with little staining reaction in the other parts of the hepatocytes.
- A strong, distinct staining of the Burkitt lymphoma and the clear cell renal carcinoma.
- At least a weak to moderate staining of the high grade follicular lymphoma.
- A negative staining reaction of the B-CLL (only residual germinal centres should be positive).
- In all specimens neutrophil granulocytes should be demonstrated.

89 laboratories participated in the assessment. At the assessment 26 achieved optimal marks (29 %), 38 good (43 %), 17 borderline (19 %) and 8 (9 %) poor marks.

All laboratories used the mAb clone **56C6** from following vendors:
mAb clone **56C6** (Novocastra n=70, NeoMarkers n=6, Ventana n=6, Monosan n=3, Cell Margue n=1, Signet n=1, Vector n=1, Zymed n=1)

All protocols resulting in an optimal staining were based on HIER using either a Tris-EDTA/EGTA buffer pH 9 of which 22 out of 61 laboratories using this obtained an optimal mark or CC1 (Cell Conditioning 1, Ventana) of which 4 out of 18 obtained an optimal mark. Interestingly 6 out of 6 laboratories using HIER in Citrate pH 6 all were assessed as poor or borderline. The mAb clone **56C6** could both be used as a concentrate or as a Ready-To-Use product. In the optimal protocols using a concentrate the mAb was typically used in the range of 1:10 – 100 depending on the total sensitivity of the protocol employed. The combination of the mAb clone **56C6**, diluted in the range of 1:10 – 1:100 and HIER in Tris-EDTA/EGTA pH 9 or CC1 resulted in an optimal staining in 26 out of 71 laboratories (37 %) and an sufficient staining - optimal or good - in 57 out of 71 laboratories (80 %). Using a RTU Ab and HIER in either Tris-EDTA/EGTA pH 9 or CC1 resulted in an optimal staining in 2 out of 10 laboratories (20 %) and an sufficient staining - optimal or good - in 7 out of 10 laboratories (70 %).

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient or excessive HIER
- HIER in other buffer than Tris-EDTA/EGTA pH 9 or CC1

In the assessment the prevalent feature of an insufficient staining was a too weak or false negative staining of both the normal germinal centre B-cells in the tonsil and the neoplastic cells in both the Burkitt lymphoma but especially in the high grade follicular lymphoma. In general the majority of the laboratories were able to detect CD10 in the clear cell renal carcinoma, the bile canaliculi and in the neutrophils.

A good quality indicator was the ability to demonstrate CD10 in virtually all germinal centre cells in the tonsil. The cells should display a strong distinct continuous membranous reaction without reaction of peripheral mantle zone B-cells.

Conclusion
- The mAb clone **56C6** is an appropriate marker for CD10.
- HIER in an alkaline buffer as Tris-EDTA/EGTA pH 9 or CC1 is highly recommended for optimal performance.

CD10 was also assessed in run 6, in which 43 laboratories participated. Out of these 38 % (16 laboratories) had an insufficient staining. Each laboratory was given specific recommendations to improve their protocol. 14 laboratories, which obtained an insufficient result in run 6 submitted a new CD10 stain in run 16. 10 out of these

followed the recommendation and 9 of them (90 %) improved from insufficient to either good or optimal. 4 laboratories did not follow the recommendations and one improved from insufficient to good. The overall proportion of insufficient staining was in this run reduced from 38 % in run 6 to 28 % in run 16. Focusing only on the laboratories participating in both runs (n=40) the proportion of insufficient staining was reduced from 38 % (n=15) to 20 % (n=8).

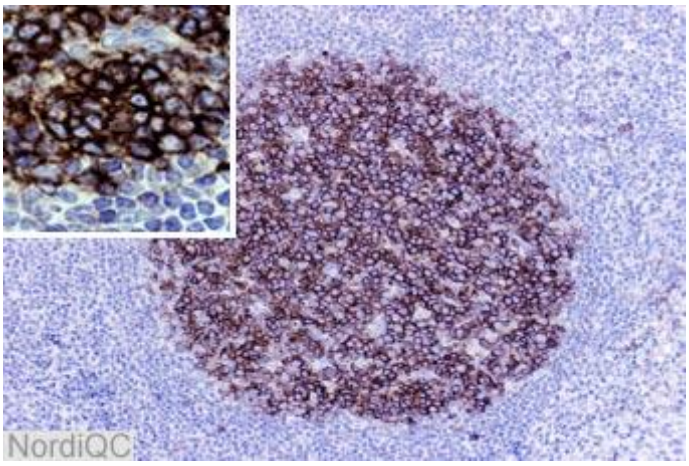

Fig. 1a
Optimal staining for CD10 of the tonsil. The germinal centre B-cells show a distinct and continuous membranous staining. There is no background staining.

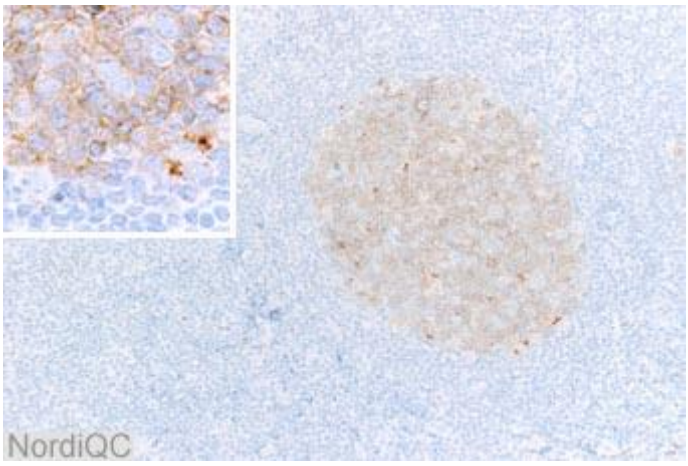

Fig. 1b
Staining for CD10 of the tonsil using an insufficient protocol (same field as in Fig. 1a.). The cells expected to cells are demonstrated, however the stain is weak and punctate. Compare with Fig. 2b – same protocol.

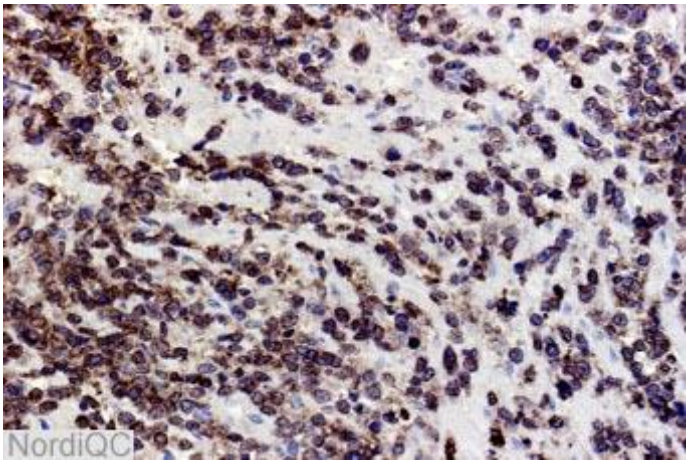

Fig. 2a
Optimal staining for CD10 of the Burkitt lymphoma. Virtually all of the neoplastic cells show a strong and distinct staining (same protocol used in Fig. 1a).

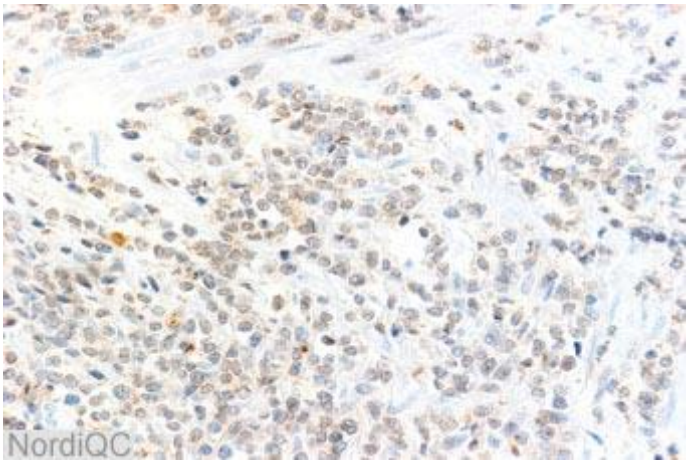

Fig. 2b
Insufficient staining for CD10 of the Burkitt lymphoma (same field as in Fig 2a). The neoplastic cells are weakly positive or false negative (same protocol used in Fig. 1b).

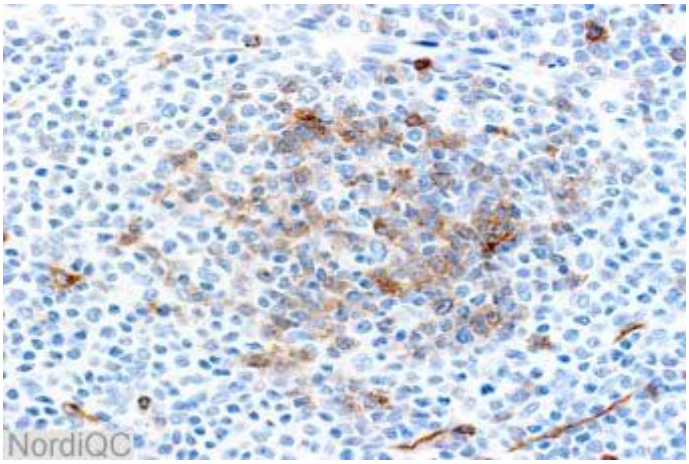

Fig. 3a
Optimal staining for CD10 of the high grade follicular lymphoma. The majority of the neoplastic cells show a moderate and distinct staining.

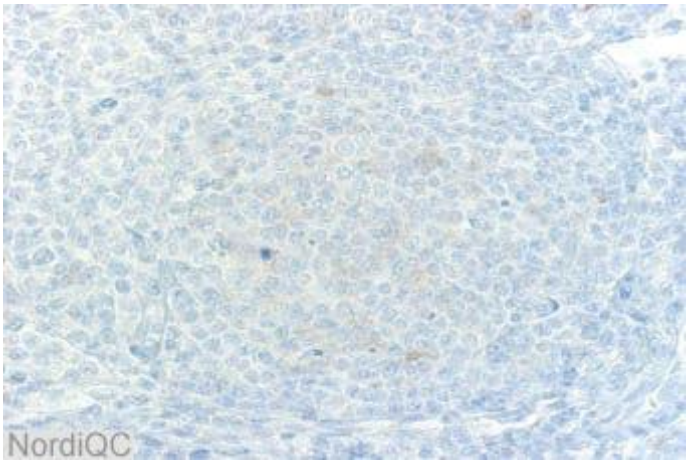

Fig. 3b
Insufficient staining for CD10 of the high grade follicular lymphoma (same field as in Fig 2a). The neoplastic cells are weakly positive or false negative.

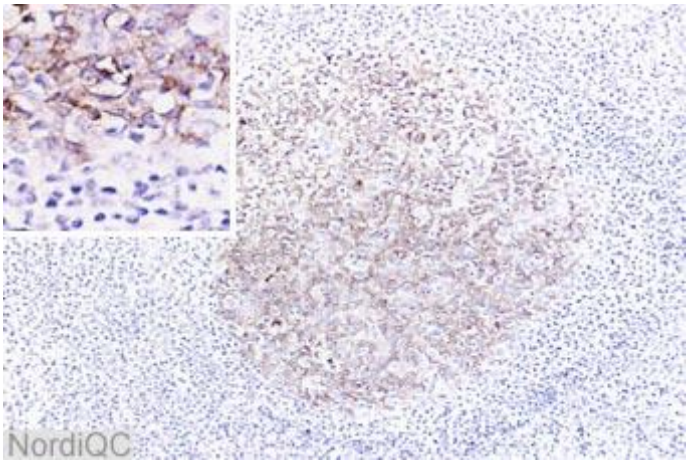

Fig. 4a
Insufficient staining for CD10 in the tonsil (same field as in Fig. 1a). The germinal centre B-cells show an impaired morphology and imprecise localization of CD10, most likely due to excessive heat induced epitope retrieval.

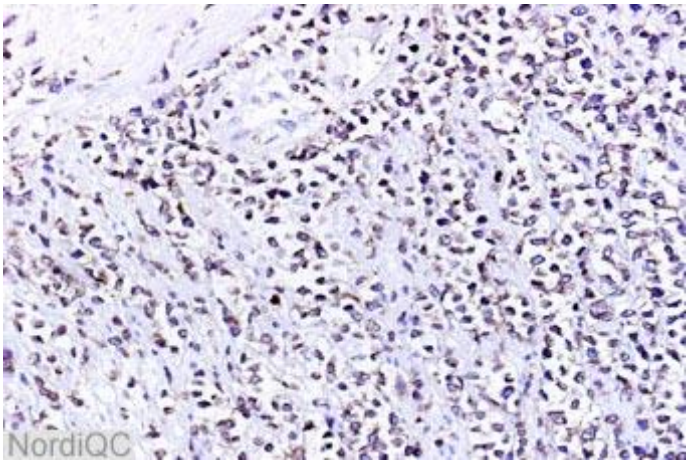

Fig. 4b
Insufficient staining for CD10 in the Burkitt lymphoma (same field as in Fig 2a). All the neoplastic cells are virtually negative and show an impaired morphology (same protocol used in Fig. 4a).

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