

The slides to be stained for CD10 contained four small lymphocytic lymphomas/chronic lymphocytic leukaemias (specimens 1-4), two normal kidneys (specimens 5-6), a colon carcinoma (specimen 7), three Hodgkin lymphomas LP (specimens 8-10), and three follicular lymphomas (specimens 11-13).



43 laboratories submitted stainings. All laboratories used mAb 56C6 from either Novocastra (most), Neomarkers or Ventana. All used HIER as pre-treatment.

At the assessment, 14 laboratories (33%) achieved optimal staining, 13 (30%) acceptable, 11 (26%) borderline, and 5 (12%) poor. The basis for an optimal result was an intense and distinct staining of cell membranes in cells expected to stain.

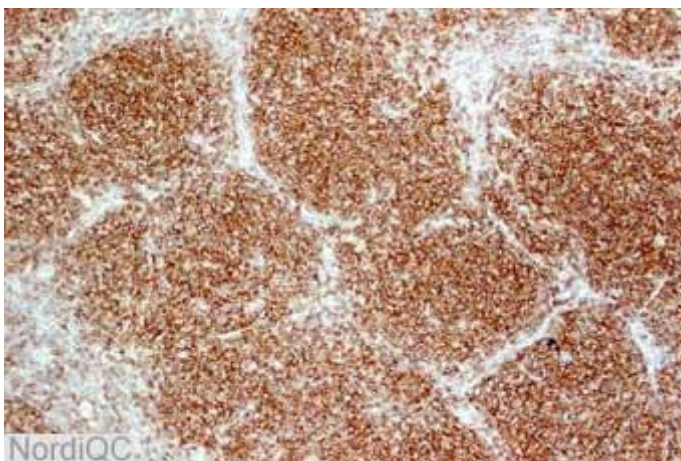
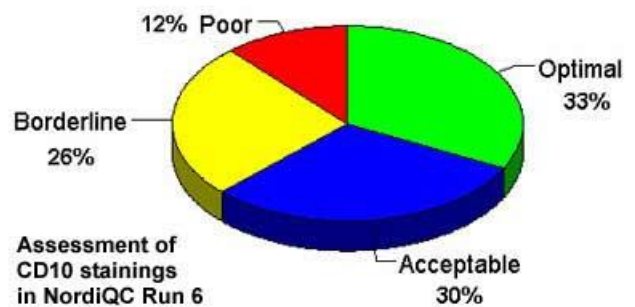
Mandatory for an optimal CD10 staining reaction was an efficient HIER protocol combined with a sensitive visualization system and a method to avoid endogenous biotin reaction.

All laboratories achieving an optimal demonstration of CD10 used HIER in an alkaline buffer (10/1 mM Tris/EDTA or Tris/EGTA), and 13 of 14 used a polymer based visualization system (EnVision), while one used an avidin/biotin system with an efficient blocking of endogenous biotin.

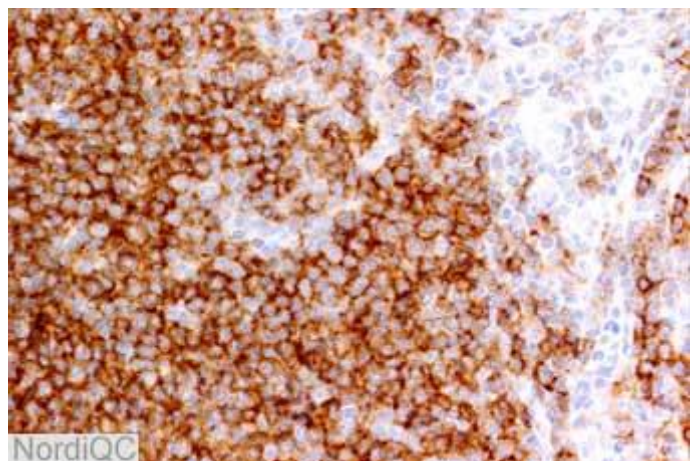
The most frequent reasons for an insufficient staining were:

- insufficient HIER (citrate pH 6),
  - too dilute primary Ab concentration,
  - HIER with an alkaline buffer and an avidin-biotin based visualization system without endogenous biotin blocking.
- A too weak staining was particularly obvious in lymphatic germinal centres.

A normal lymph node is appropriate for control tissue. Care should be taken that almost all germinal centre cells show a distinct membranous staining.



**Fig. 1a**  
Optimal staining using mAb 56C6. Follicular lymphoma showing intense staining of most cells in the neoplastic follicles. x100



**Fig. 1b**  
Higher magnification of staining shown in Fig. 1a. Distinct membranous staining of most neoplastic B-cells. x400.

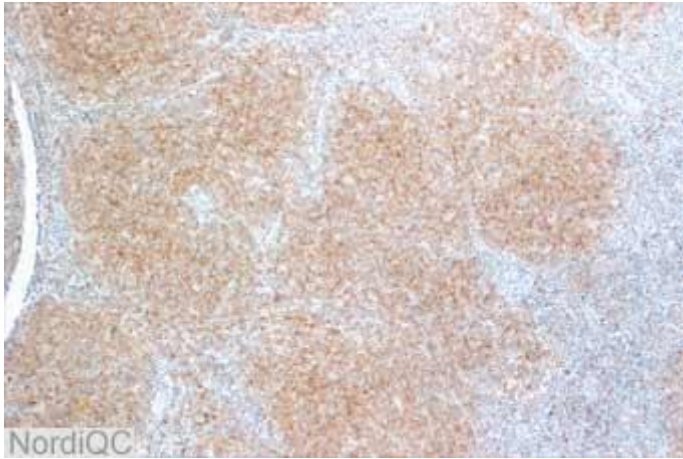


Fig. 2a  
Acceptable staining. Same Ab and same field as in Fig. 1.  
x100.

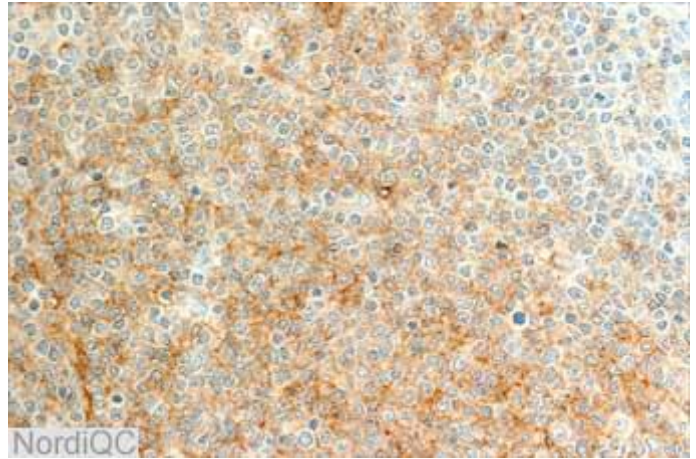


Fig. 2b  
Higher magnification of staining shown in Fig. 2a. Weaker and  
less distinct staining than in Fig. 1b but acceptable for  
diagnostic purposes. x400.

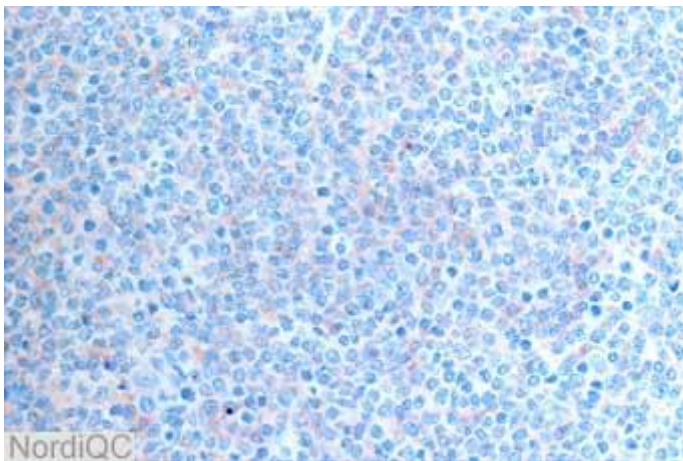


Fig. 3  
Insufficient staining. Same Ab and same field as in Fig. 1 and  
2. x 400. The neoplastic cells are either weakly stained or  
unstained.

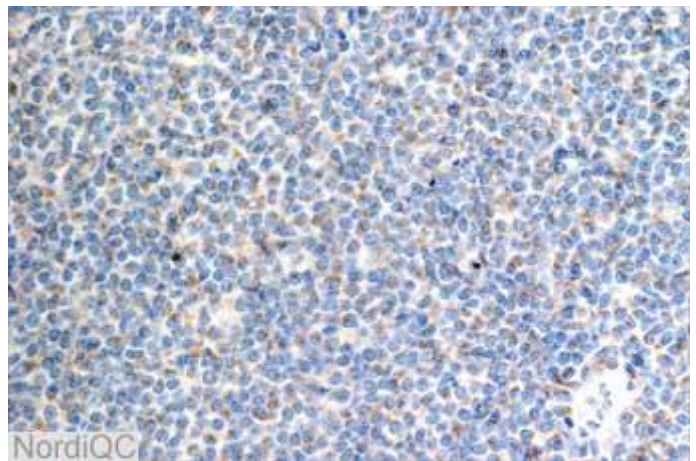


Fig. 4  
Insufficient staining of small lymphocytic lymphoma. False  
positive staining of most neoplastic cells, probably due to  
endogenous biotin, which was not suppressed in this protocol  
based on HIER in an alkaline buffer and an avidin-biotin based  
system (compare with Fig. 5b).



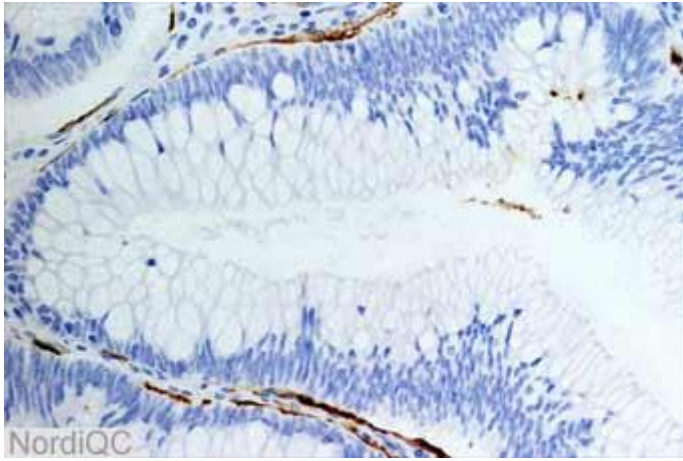


Fig. 5a  
Colon adenocarcinoma stained for CD10 (mAb 56C6) using a good protocol. Only the stromal myofibroblastic cells are stained.

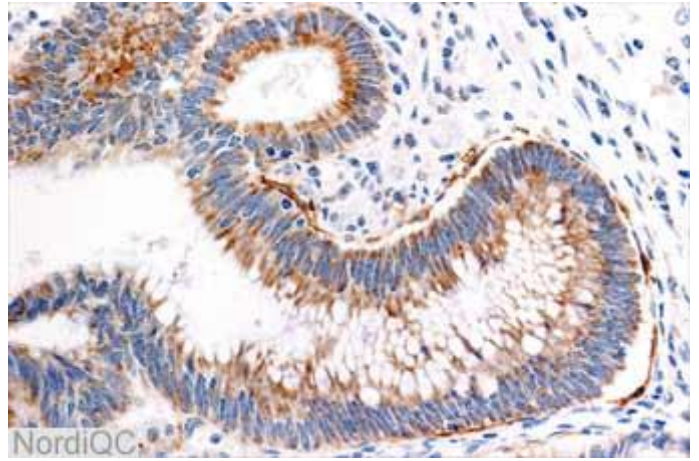


Fig. 5b  
Same Ab and field as in Fig. 5a, using an insufficient protocol (same as illustrated in Fig. 4). The myofibroblasts are appropriately stained, but a strong false positive staining of the enterocytes is also seen.

SN/MV/LE 16-6-2004