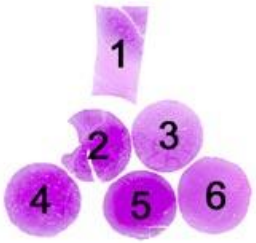
Assessment Run 21 2007
CD20

The slide to be stained for CD20 comprised:
1. Appendix, 2. Tonsil, 3. Follicular lymphoma, 4. Precursor-B-ALL (testis), 5. B-CLL, 6. Plasmacytoma.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a CD20 staining as optimal included:

- A strong, predominantly membranous staining of the mantle zone B-cells, the germinal centre B-cells and the interfollicular B-cells in the tonsil and the appendix.
- A strong membranous staining of virtually all the neoplastic cells of the follicular lymphoma and the B-CLL.
- A negative staining of the Precursor-B-ALL (only scattered maturated neoplastic cells and entrapped normal B-cells may be demonstrated).
- A negative staining of the plasmacytoma (only the remnants of normal B-cells should be demonstrated).
- A negative staining of all other cell types.

115 laboratories submitted stains. At the assessment 66 achieved optimal marks (57 %), 34 good (30 %), 12 borderline (10 %) and 3 poor marks (3 %).

The following Abs were used:
mAb clone **L26** (Dako, n=99, Ventana n=11, BioGenex n=1, NeoMarkers n=1, Zymed n=1)
mAb clone **7D1** (Novocastra n=1)
pAb **RN-9013** (NeoMarkers n=1)

Optimal staining for CD20 in this assessment was obtained with the mAb clone **L26** (65 out of 113) and the pAb **RN-9013** (1 out of 1).

L26: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (31/59)*, Cell Conditioning1 (BenchMark, Ventana) (15/24), Citrate pH 6 or 7 (7/11), EDTA/EGTA pH8 (3/4), Target Retrieval Solution pH 6,1 (TRS, Dako) (3/3), Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) (2/4), Bond Epitope Retrieval Solution 1 (Bond, Vision Biosystems)(1/1), 1mM EDTA pH 9 (1/1) or PT Module Buffer 1 pH 6 (LabVision) (1/1) as HIER buffer. The mAb was typically diluted in the range of 1:50 – 1:3,000** depending on the total sensitivity of the protocol employed, or as a Ready-To-Use (RTU) antibody. Using these protocol settings 95 out of 108 (88 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)
** Dako has changed the anti-CD20 Ig concentration recently, which explains the wide range of Ab concentration

RN-9013: The protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 and a dilution of 1:1.000 of the primary Ab.

The most frequent causes of insufficient staining were
- Too low concentration of the primary antibody
- Omission of HIER.

The prevalent feature of the insufficient results was a general too weak and diffuse staining of both the normal and neoplastic cells supposed to be demonstrated, mainly related to a too low concentration of the primary Ab resulting in an imprecise and patchy staining of the membranes especially in the mantle zone B-cells and the the B-CLL.
Tonsil is appropriate for control tissue: the staining of the B-cells should be as strong as possible with no reaction in other cells (epithelial cells, muscle cells etc.).
HIER is mandatory to obtain an optimal reaction. Efficient HIER in an alkaline buffer and/or with the use of a pressure cooker typically allowed the laboratories to use a lower concentration of the clone L26 compared to a

milder HIER in a Citrate buffer pH 6. However, the use of the milder HIER seemed to be beneficial to the morphology and resulted in a very crisp and precise localization of CD20. The difference in HIER did not affect the interpretation.

CD20 was also assessed in run 6 2002, where 62 laboratories participated. Even though the number of participants has almost doubled, the results of the 2 runs are comparable, as the proportion of sufficient stains increased from 81% t o 87%.

Conclusion
The mAb clone **L26** and the pAb **RN-9013** are useful for the demonstration of CD20. HIER is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated. Tonsil is an appropriate control: The mantle zone B-cells and the germinal centre B-cells must show a strong reaction. No other cells should stain.

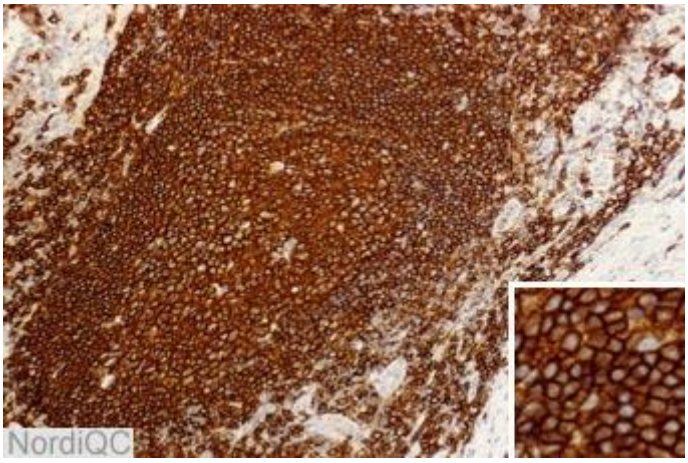


Fig. 1a
Optimal CD20 staining of the tonsil using the mAb clone L26. Virtually all the mantle zone B-cells, the germinal centre B-cells and the interfollicular B-cells show a strong and distinct staining. Insert: High magnification of the mantle zone showing the crisp continuous membrane staining of the B-cells.

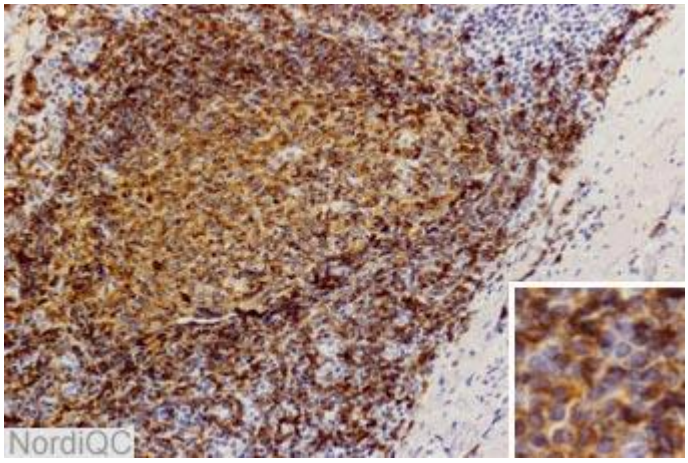


Fig. 1b
CD20 staining of the tonsil – same field as in Fig. 1a – using an insufficient protocol based on the mAb clone L26 (too low concentration). At low magnification the B-zone is demonstrated. Insert: High magnification of the mantle zone revealing a diffuse reaction of the B-cells. Also compare with Fig. 2b – same protocol.

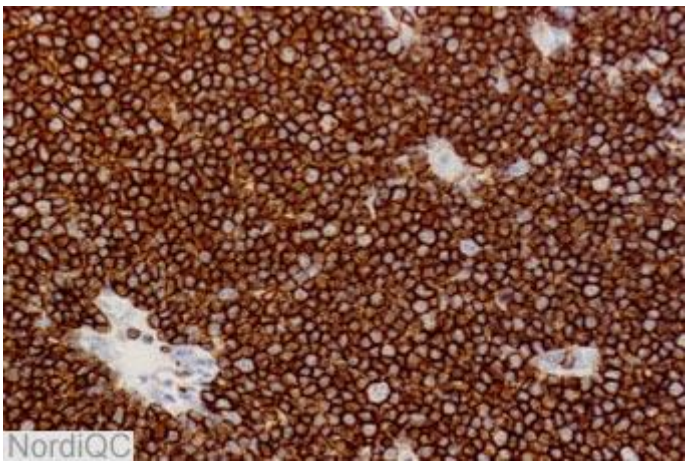


Fig. 2a
Optimal CD20 staining of the B-CLL using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong distinct reaction with no background reaction.

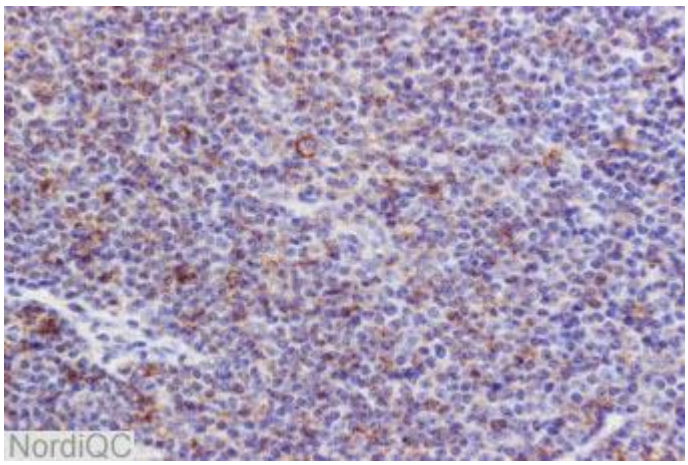


Fig. 2b
Insufficient CD20 staining of the B-CLL using same protocol as in Fig. 1b. Only scattered neoplastic cells show a weak reaction – same field as in Fig. 2a.

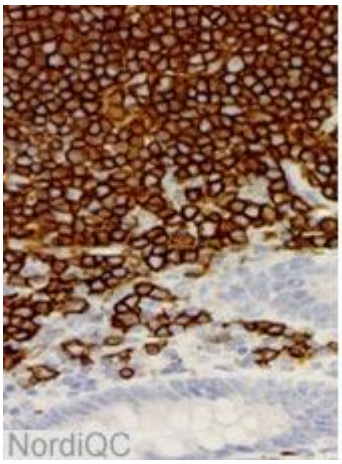


Fig. 3a
Optimal CD20 staining using HIER in Citrate pH 6.0 and pressure cooker. The normal and malignant B-cells show a crisp and distinct membranous reaction.
Left: Appendix. Right: Follicular lymphoma

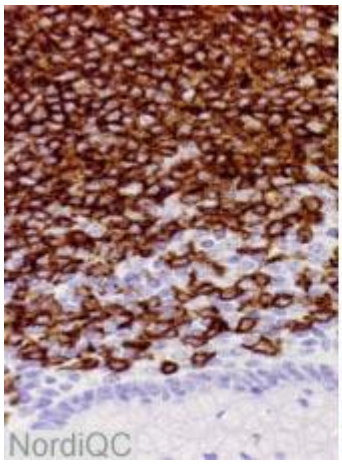


Fig. 3b
Optimal CD20 staining using HIER in Tris-EDTA pH 9.0 and pressure cooker. The normal and malignant B-cells show a strong staining, but a more coarse reaction than in Fig. 3a. No difference was seen in the proportion of positive cells using the two HIER procedures, when optimized.
Left: Appendix. Right: Follicular lymphoma

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