Assessment Run 19 2007
CD23

The slide to be stained for CD23 comprised:
1-3. Tonsils fixed 4h, 72h, and 168h, resp., 4-5. Chronic lymphatic leukaemia (B-CLL), 6. Mantle cell lymphoma (MCL).
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD23 staining as optimal included:

- A strong, distinct membranous staining of the activated B-cells in the mantle zone of the germinal centres in the tonsils.
- A strong, distinct staining of the follicular dendritic cells in the germinal centres.
- A strong, distinct membranous staining of majority of the neoplastic cells in the two B-CLLs.
- No staining of MCL.

88 laboratories submitted stains. At the assessment 25 achieved optimal marks (28 %), 23 good (26 %), 29 borderline (33 %) and 11 poor marks (13 %).
The following Abs were used:
mAb clone **1B12** (Novocastra, n=56; Ventana, n=5; Cell Marque, n=3; Monosan, n=2; NeoMarkers, n=2)
mAb clone **BU38** (The Binding Site, n=2)
mAb clone **MHM6** (Dako, n=10)
rmAb **SP23** (NeoMarkers, n=8)

Optimal staining for CD23 in this assessment was obtained with the mAb clone **1B12** (21 out of 68; 31%) and the rmAb clone **SP23** (4 out of 8; 50%).

1B12: The protocols giving optimal results were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Tris-EDTA/EGTA pH 9, Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) or Cell Conditioning1 (BenchMark, Ventana). The mAb was typically diluted in the range of 1:20 – 1:200 depending on the total sensitivity of the protocol employed. Using these settings 39 out of 51 laboratories (76 %) produced a sufficient staining (optimal or good).
Only 1 of 9 protocols based on the use of **1B12** as a Ready-To-Use (RTU) Ab gave a sufficient staining result, despite the protocol settings being otherwise identical to the those based on a concentrated Ab giving sufficient results.
SP23: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 as buffer. The Ab was diluted in the range of 1:100 – 1:200 depending on the total sensitivity of the protocol employed. Using these settings all of 7 laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Less successful primary Ab
- Less successful RTU Ab clone 1B12
- Too low concentration of the primary Ab
- HIER in non-alkaline buffer (typically Citrate pH 6.0)

In the assessment virtually all laboratories were able to demonstrate CD23 in the follicular dendritic network within the germinal centres, whereas the prevalent feature of an insufficient staining was a too weak or false negative staining of the mantle zone B-cells and neoplastic B-cells in the two B-CLLs. In general, CD23 is only weakly expressed in B-CLL and a sensitive protocol is required to demonstrate CD23 properly.
Normal tonsil was found to be a reliable quality indicator for the immunohistochemical demonstration of CD23, as the sufficient results all showed a distinct membranous reaction of the activated mantle zone B-cells. If these B-cells were negative or only weakly demonstrated, the B-CLLs also were negative or only equivocally stained.

CD23 was also assessed in Run 9 2003, in which 57 laboratories participated.
The overall proportion of sufficient staining decreased from 76 % in run 9 to 55 % in the present run. This is probably due to several factors including many new participants, more challenging tissue material circulated, and a higher proportion of the laboratories using 1B12 as an RTU product.
Including only the laboratories participating in both runs (n=56) the proportion of sufficient staining decreased from 75 % 66 %.
13 laboratories, which obtained an insufficient result in run 9, submitted a new CD23 stain in run 19. 10 of them followed the recommendations and 7 improved their result to good or optimal (70 %). 3 laboratories did not follow the recommendations and 1 of these (33 %) obtained a sufficient staining in run 19.

The choice of Ab clone and format (concentrated v. RTU) had a high impact on the final assessment result. Only the concentrated format of clones **1B12** and **SP23** (i.e., the dilution is calibrated by the laboratories) could give optimal results. It is particularly noteworthy that none of 10 laboratories using clone MHM6 obtained an optimal result in this run (in run 9, 2 out of 8 was optimal).

Conclusion

The mAb clones **1B12** and **SP23** are useful for the detection of CD23 in B-cell lymphomas. HIER in an alkaline buffer (Tris-EDTA/EGTA pH 9, BERS2 or CC1) is mandatory for optimal performance.
The current RTU Abs have not been calibrated properly to fulfil the diagnostic demands. The Ab concentration in the RTU format must be related to the total sensitivity of the protocol employed, and this sensitivity has to be defined in a clinical setting and validated by the producer as well as the laboratories.
A tonsil/lymph node is appropriate control: The activated mantle zone B-cells must show a distinct membranous reaction. It has to be emphasized that the follicular dendritic cells shall not be used as positive control for CD23, as these cells strongly express CD23 and can consequently not be used to calibrate the immunohistochemical protocol.

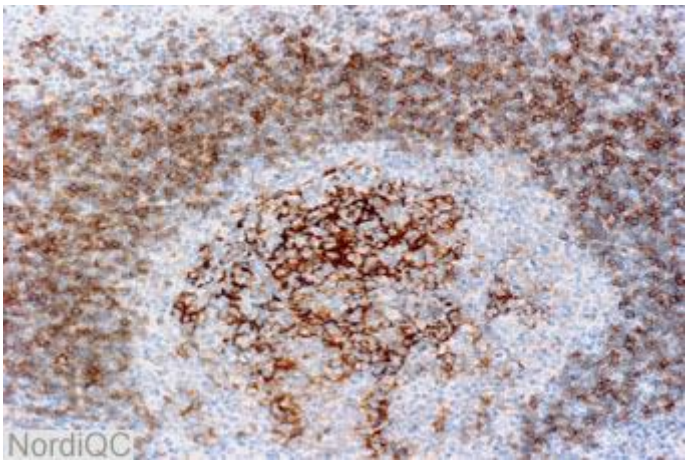

Fig. 1a
Optimal staining for CD23 of a secondary follicle in the tonsil using the mAb clone 1B12 appropriately calibrated. Both the follicular dendritic cells and the activated mantle zone B-cells show a strong and distinct staining.

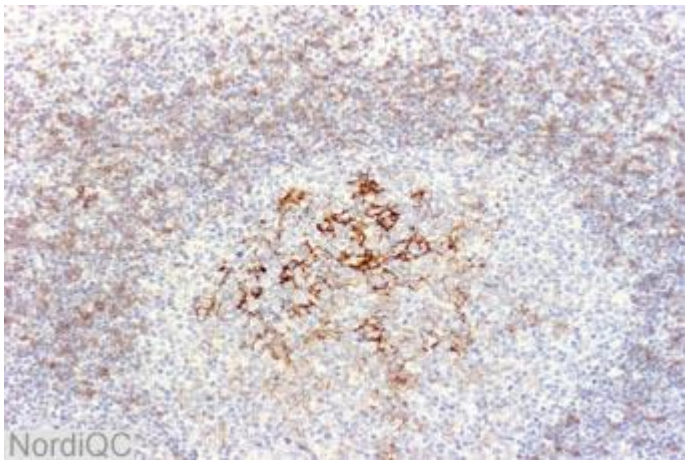

Fig. 1b
Staining for CD23 of a secondary follicle in the tonsil using an insufficient protocol (same field as in fig 1a).
Both the proportion of positive cells and the staining intensity is reduced compared to Fig. 1 a. The protocol was based on the clone 1B12, but in a too low concentration.

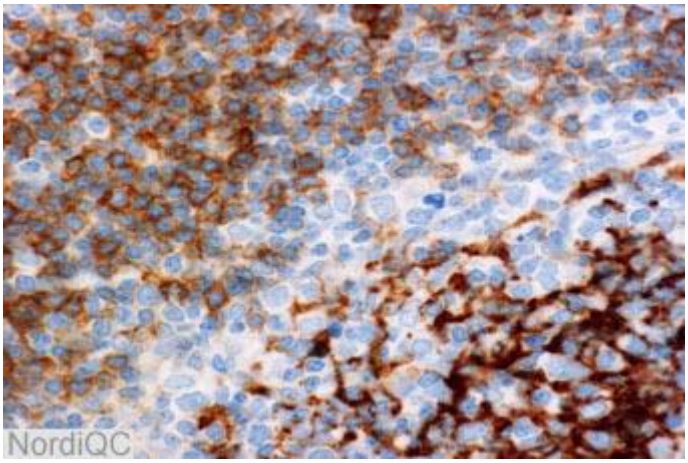

Fig. 2a
High magnification of the optimal staining in Fig 1a of the secondary follicle in the tonsil. The activated B-cells show a distinct continuous membranous reaction.

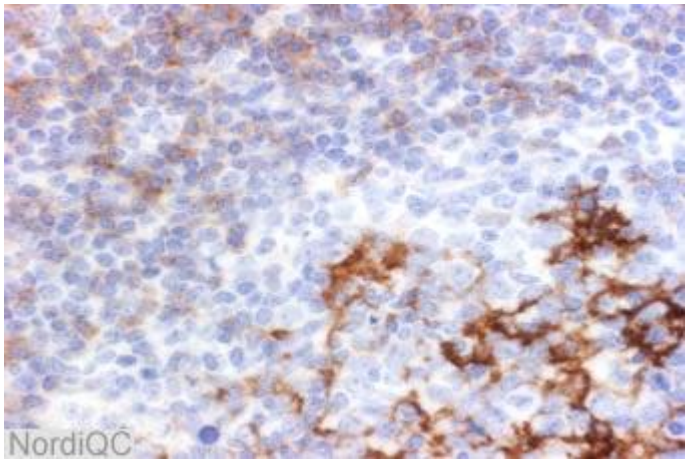

Fig. 2b
High magnification of the insufficient staining in Fig 1b of the secondary follicle in the tonsil (same field as in Fig 2a). The activated B-cells only show a weak imprecise reaction.

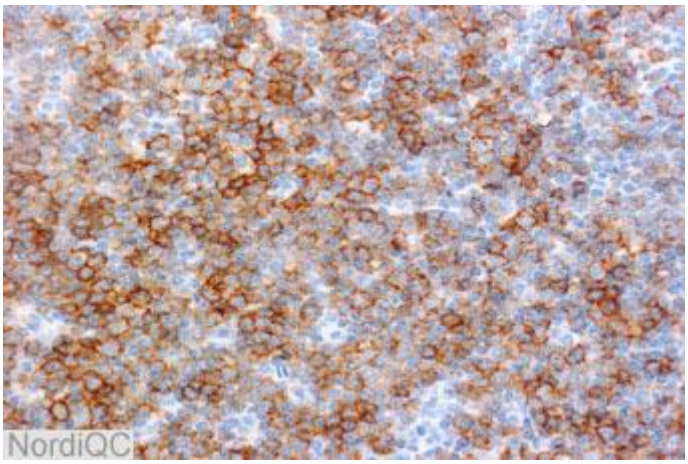

Fig. 3a
Optimal staining for CD23 of the B-CLL no 4 using same protocol as in Fig 1b and 2 b. The majority of the neoplastic cells show a strong and distinct membranous staining.

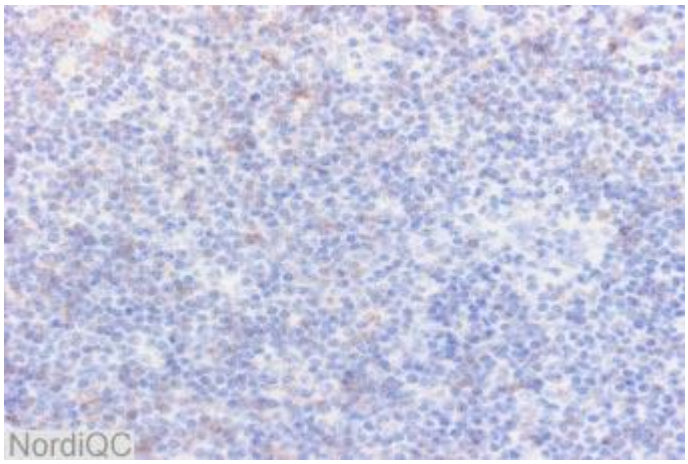

Fig. 3b
Insufficient staining for CD23 of the B-CLL no 4 using same protocol as in Fig 1b and 2 b. The neoplastic cells are virtually negative.

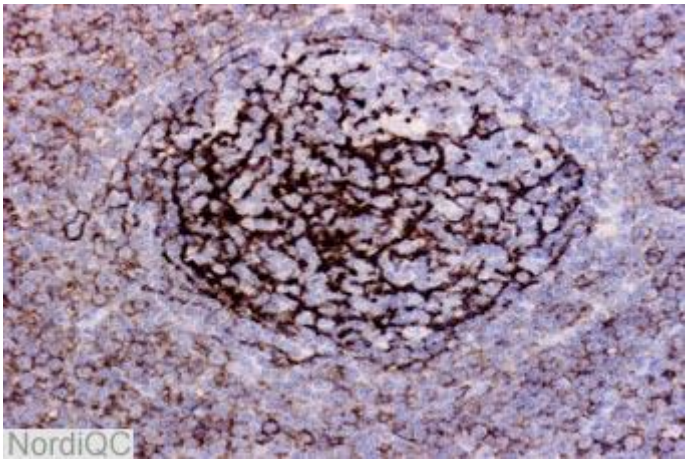

Fig. 4a
Optimal staining for CD23 of the B-CLL no 5 using mAb clone SP23. The majority of the neoplastic cells show a moderate and distinct membranous staining, while remnants of the normal follicular dendritic cells entrapped show a strong staining.

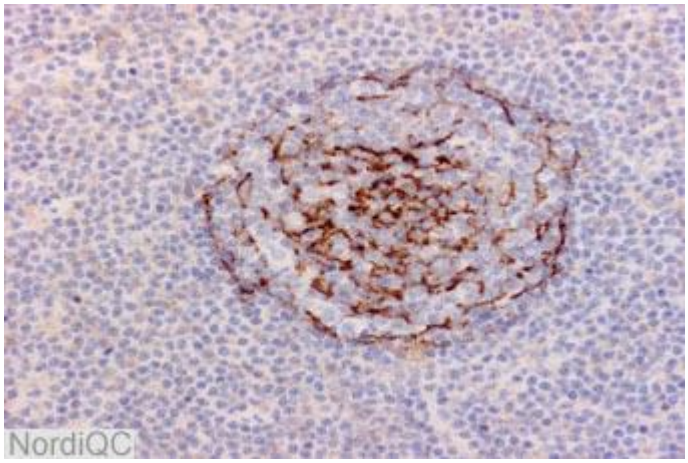

Fig. 4b
Insufficient staining for CD23 of the B-CLL no 5 using mAb clone MHM6 (same field as in Fig. 4a). The neoplastic cells are all negative, and only the remnants of the normal follicular dendritic cells are demonstrated.

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