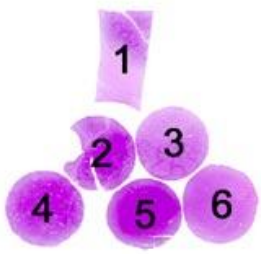
Assessment Run 21 2007

CD79a



The slide to be stained for **CD79a** comprised:
1. Appendix, 2. Tonsil, 3. Follicular lymphoma, 4. Precursor-B-acute lymphatic leukaemia (ALL; testis), 5. B-chronic lymphatic leukaemia (CLL), 6. Plasmacytoma. All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD79a staining as optimal included:

- A strong, predominantly membranous staining of the mantle zone B-cells and a moderate to strong membranous staining of the germinal centre B-cells in the secondary follicles in the tonsil and the appendix.
- A strong, predominantly cytoplasmic reaction in the plasma cells and the late stage activated germinal centre B-cells in the tonsil and the appendix.
- A moderate to strong membranous staining of the majority of the neoplastic cells of the follicular lymphoma, B-CLL and the Precursor-B-ALL.
- A moderate to strong cytoplasmic reaction in the majority of the neoplastic cells of the plasmacytoma.
- No staining of any other cells.

112 laboratories submitted stains. At the assessment 56 achieved optimal marks (50 %), 31 good (28 %), 13 borderline (12 %) and 12 poor marks (11 %).

The following Abs were used:
mAb clone **JCB117** (Dako, n=88; Ventana, n=6; NeoMarkers, n=3)
mAb clone **HM57** (Dako, n=6)
mAb clone **11E3** (Novocastra, n=2))
mAb clone **HM47/A9** (Monosan, n=1)
rmAb clone **SP18** (NeoMarkers, n=6)

Optimal staining for CD79a in this assessment was obtained with the mAb clone **JCB117** (51 out of 97) and the rmAb **SP18** (5 out of 6).

JCB117: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (33/52)*, Cell Conditioning 1 (BenchMark, Ventana) (8/23), Citrate pH 6 (6/11), EDTA/EGTA pH8 (2/6) or Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) (2/3) as heating buffer. The mAb was typically diluted in the range of 1:20 – 1:700 depending on the total sensitivity of the protocol employed or as a Ready-To-Use (RTU) antibody. Using these protocol settings 77 out of 91 (85 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

SP18: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (2)**, Cell Conditioning 1 (BenchMark, Ventana) (1), 1mM EDTA pH 9 (1) or Citrate pH 6 (1) as HIER buffer. The Ab was diluted in the range of 1:100 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining.
** (number of laboratories using this buffer)

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Insufficient HIER (too short heating time)

In this assessment the prevalent feature of an insufficient staining was a too weak or negative reaction of cells expected to be demonstrated. The majority of the laboratories were able to demonstrate CD79a in the mantle zone B-cells as well as in the follicular lymphoma and the B-CLL. However, the demonstration of CD79a in both the precursor-B-ALL and the plasmacytoma was much more difficult and only obtained when using protocols with a high sensitivity (including efficient HIER) and a primary Ab with high affinity for CD79a, i.e. JCB117 or SP18. Clone HM57 appeared to have a too low affinity for CD79a in both the precursor-B-ALL and the plasmacytoma, as

all 6 out of 6 protocols based on this clone gave a weak or false negative reaction. At the same time HM57 gave a strong cross-reaction with smooth muscle cells and the appendiceal columnar epithelial cells. It should be emphasized that clone HM57 by Dako is recommended especially for the identification of the CD79α-equivalent protein in various mammals but **not** in human tissue. Clone HM47/A9 gave the same poor staining pattern as HM57. Clone 11E3 gave a distinct reaction in the mantle zone B-cells and plasma cells, but only faintly with the germinal centre cells and false negative in the plasmacytoma and precursor-B-ALL. Only two laboratories used 11E3, but as all other protocol parameters were identical to those used to obtain optimal results with JCB117 and SP18, it appears that 11E3 may have a lower affinity for CD79a.

Appendix is a reliable positive control: The germinal centre B-cells must show a moderate to strong reaction. If only the mantle zone B-cells is distinctively demonstrated, the protocol may be too insensitive to detect CD79a in the Precursor-B-ALL and the plasmacytoma. The appendiceal epithelial cells and smooth muscle cells should be negative.

This was the second run of CD79a, as the marker also was assessed in run 6 2002, where 52 laboratories participated. Even though the number of participants has doubled, the results of the two runs are comparable: The proportion of sufficient stains was 83% and 78%, respectively. Also in run 6 it was observed, that clone HM57 was inappropriate for the demonstration of CD79a.

Conclusion
The mAb clone **JCB117** and the rmAb **SP18** are both useful for the demonstration of CD79a. HIER is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated. Appendix and tonsil are appropriate controls: The germinal centre B-cells must show a moderate to strong staining reaction.

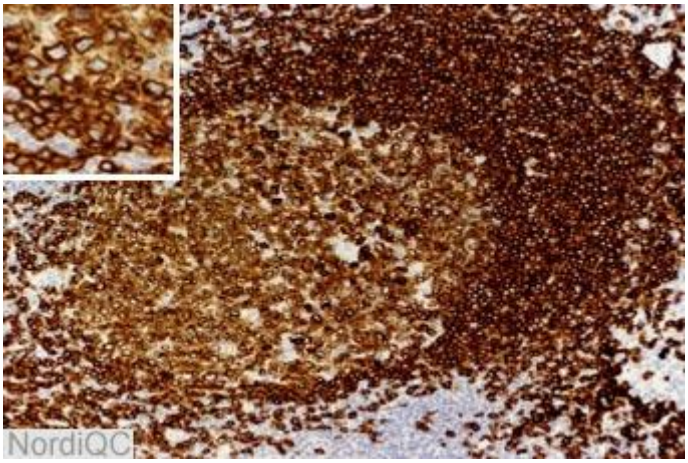


Fig. 1a
Optimal CD79a staining of the tonsil using the mAb clone JCB117. The mantle zone B-cells show an intense staining, while the germinal centre B-cells show a moderate staining. Insert high magnification of the mantle zone showing the difference in staining intensity of the two cell types.

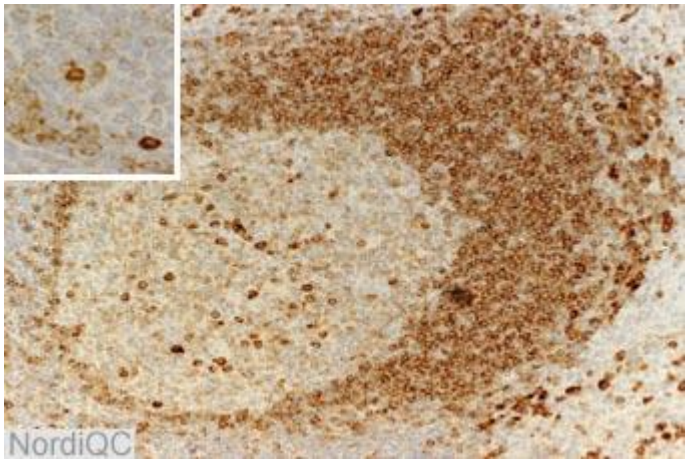


Fig. 1b
CD79a staining of the tonsil – same field as in Fig. 1a – using an insufficient protocol based on the mAb clone JCB117 (too low concentration). At low magnification only the mantle zone B-cells and the late stage germinal centre B-cells are distinctively demonstrated. Insert at high magnification of the mantle zone showing the diffuse reaction of the germinal centre B-cells. Also compare with Fig. 2b & 3b – same protocol.

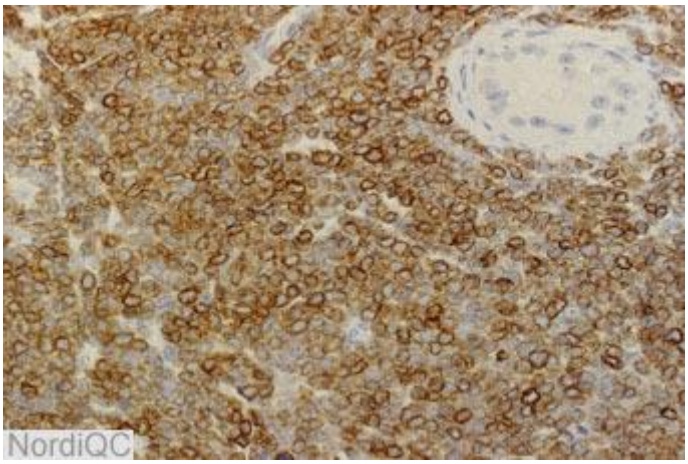


Fig. 2a
Optimal CD79a staining of the precursor B-ALL using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a distinct reaction with no background reaction.

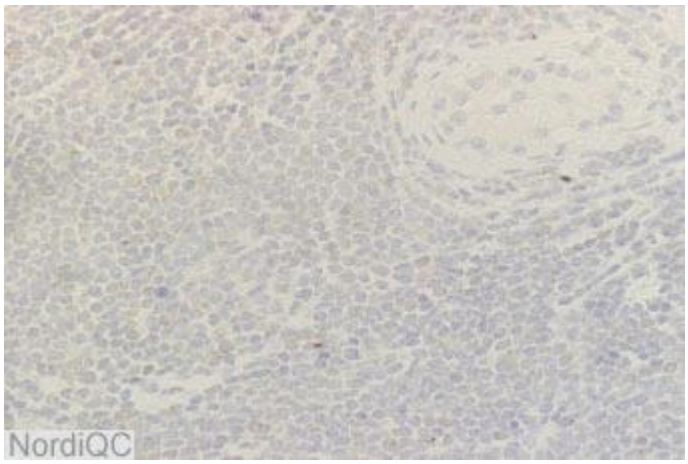


Fig. 2b
Insufficient CD79a staining of the precursor B-ALL using same protocol as in Fig. 1b. The neoplastic cells are negative – same field as in Fig. 2a.

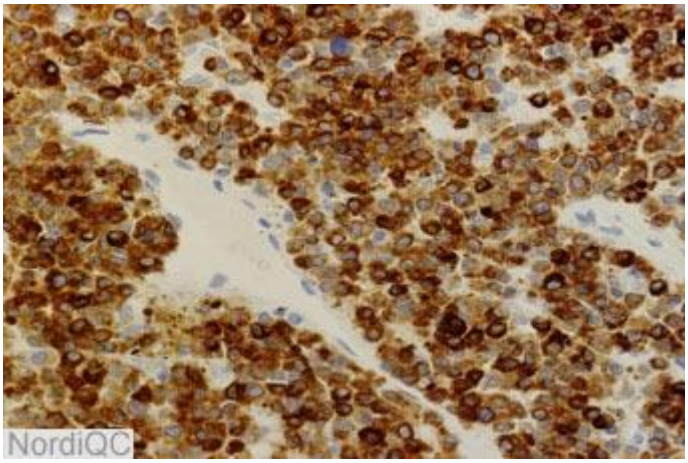


Fig. 3a
Optimal CD79a of the plasmacytoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic reaction with no background reaction.

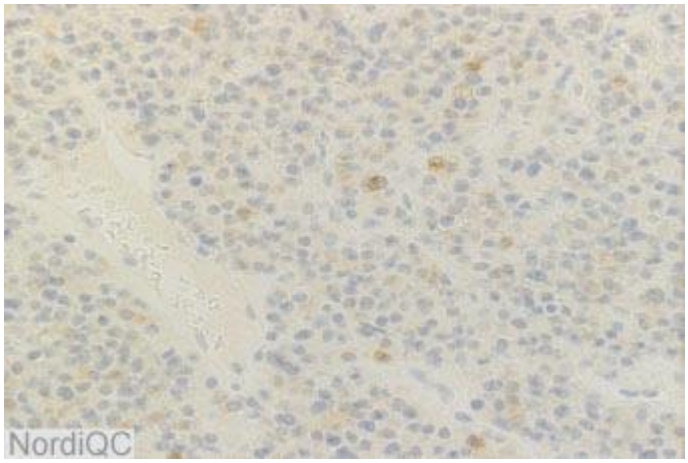


Fig. 3b
Insufficient CD79a of the plasmacytoma using same protocol as in Fig. 1b & 2b. Only scattered neoplastic cells show a weak diffuse staining – same field as in Fig. 3a.

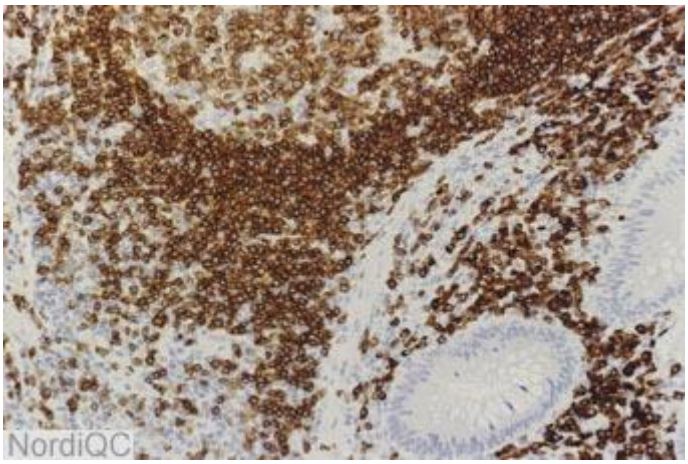


Fig. 4a
Staining of appendix using the mAb clone JCB117. The B-cells show a strong and distinct reaction and the epithelial and smooth muscle cells are negative. Compare with Fig. 4b – same field using the mAb clone HM57.

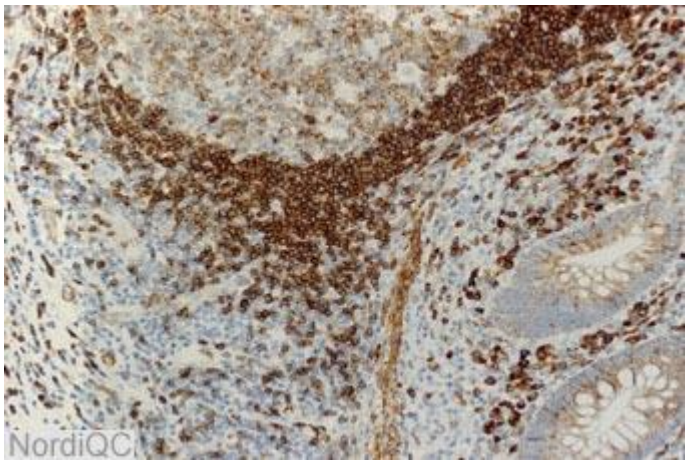


Fig. 4b
Staining of appendix using the mAb clone HM57. The mantle zone B-cells show a strong and distinct reaction, while the germinal centre B-cells are only weakly labelled. The epithelial and smooth muscle cells are also demonstrated. Compare with Fig. 4a – same field using the mAb clone JCB117.

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