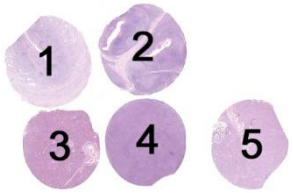
Assessment Run 29 2010
CD79a

The slide to be stained for CD79a comprised:

1. Appendix, 2. Tonsil, 3. Precursor-B-acute lymphatic leukaemia (Pre-B-ALL), 4. B-chronic lymphatic leukaemia (B-CLL), 5. Plasmacytoma.
All tissues were fixed in 10% neutral buffered formalin for 24-48 h.



Criteria for assessing a CD79a staining as optimal included:

- A strong, predominantly membranous staining reaction of the mantle zone B-cells and at least a moderate membranous staining reaction of the germinal centre B-cells in the secondary follicles of the tonsil and the appendix.
- A strong, predominantly cytoplasmic staining reaction in the plasma cells and the late stage activated germinal centre B-cells of the tonsil and the appendix.
- A moderate to strong membranous staining reaction of virtually all the neoplastic cells in the B-CLL.
- At least a weak to moderate predominantly membranous staining reaction in the majority of the neoplastic cells in the Pre-B-ALL.
- At least a weak to moderate cytoplasmic staining reaction in the majority of the neoplastic cells in the plasmacytoma.
- No staining of any other cells.

141 laboratories participated in this assessment. 75 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD79a, run 29**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb[3] clone **JCB117**	84 3 1	Dako NeoMarkers Monosan	43	32	9	4	85 %	88 %
mAb clone **HM57**	8	Dako	0	0	5	3	0 %	-
mAb clone **11D10**	1	Novocastra/Leica	0	0	0	1	-	-
rmAb clone **SP18**	8 2 1	NeoMarkers Spring Bioscience Master Diagnostica	7	3	1	0	91 %	100 %
Ready-To-Use Abs								
mAb clone **JCB117, IR621**	13	Dako	10	3	0	0	100 %	100 %
mAb clone **JCB117, 760-2630**	12	Ventana	0	4	6	2	33 %	-
mAb clone **JCB117, 179M-17**	1	Cell Marque	0	0	1	0	-	-
mAb clone **11E3, PA0192**	2	Novocastra/Leica	0	0	1	1	-	-
mAb clone **HM47/A9, PM067**	1	Biocare	0	0	1	0	-	-
rmAb clone **SP18, 760-4432**	3	Ventana	3	0	0	0	-	-
rmAb clone **SP18, ZA-0293**	1	Unknown (ZHI?)	1	0	0	0	-	-
Total	141		64	42	24	11	-	-
Proportion			45 %	30 %	17 %	8 %	75 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **JCB117**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (11/19)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (17/23), Target Retrieval Solution pH 6.1 (S1699, Dako) (1/1), Cell Conditioning 1 (BenchMark, Ventana) (3/22), Bond Epitope Retrieval Solution 2 (Bond, Leica) (7/10), EDTA/EGTA pH8 (1/1) or Citrate pH 6 (3/7) as the retrieval buffer. The mAb was typically diluted in the range of 1:25– 1:600 depending on the total sensitivity of the protocol employed. Using these protocol settings 65 out of 74 (88 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffe)

rmAb clone **SP18**: The protocols giving an optimal result were based on HIER using Tris-EDTA/EGTA pH 9 (2/3), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako)(1/1), Target Retrieval Solution pH 6.1 (S1699, Dako) (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (3/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:100– 1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 10 (100 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **JCB117** (prod. no IR621, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 12 (100 %) laboratories produced a sufficient staining.

rmAb clone **SP18** (prod. no. 760-4432, Ventana), The protocols giving an optimal result were based on HIER using standard Cell Conditioning 1, an incubation time of 16-48 min in the primary Ab and UltraView (760-500) as the detection system. Using these protocol settings all of 3 laboratories produced an optimal staining.

rmAb clone **SP18** (ZA-0293, Unknown - ZHI?), the protocol giving an optimal result was bases on HIER in Citrate pH 6 in a pressure cooker, an incubation time of 60 min in the primary Ab and a polymer based detection system (Jinqiao Zhongshan, D11-110).

The most frequent causes of insufficient staining were:

- Less successful primary antibody (e.g., all of 8 stains based the mAb clone HM57 were insufficient)
- Less successful RTU format (e.g., 9 out of 13 stains based on the mAb clone JCB117 (Ventana/Cell Marque) were insufficient)
- Too low concentration of the primary antibody
- Insufficient HIER (e.g., too short efficient heating time).

In this assessment and in concordance to the previous assessments of CD79a, the prevalent feature of an insufficient staining was a too weak or false negative reaction of cells expected to be demonstrated. The majority of the laboratories were able to demonstrate CD79a in the normal plasma cells, the mantle zone B-cells and the neoplastic cells of the B-CLL. However, the demonstration of CD79a in both the Pre-B-ALL and the plasmacytoma was much more difficult and only obtained when using correctly calibrated protocols based on mAb clone JCB117 or rmAb clone SP18 with an efficient HIER. The mAb clone HM57 has a too low affinity for CD79a, as also found in this assessment, where all of 8 protocols based on this clone gave an insufficient result. At the same time HM57 gave a strong cross-reaction with the smooth muscle cells and appendiceal enterocytes. Clone HM57, Dako, has now been withdrawn from the market. The mAb clone HM47/A9 (Biocare) gave the same poor staining pattern as clone HM57. As observed in run 21, 2007, the mAb clone 11E3 (RTU, Novocastra/Leica) gave a distinct reaction in plasma cells and the mantle zone B-cells, but only a faint staining of the germinal centre cells and virtually negative staining of the plasmacytoma and Pre-B-ALL.

Table 2. **Performance of the four most commonly used Abs in three CD79a runs**

	Run 6 2002		Run 21 2007		Run 29 2010		Total	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone JCB117	48	43	97	80	124	92	269	215 (80 %)
mAb clone HM57	3	0	6	0	8	0	17	0 (0 %)
mAb clone 11E3	0	0	2	0	2	0	4	4 (0 %)
rmAb clone SP18	0	0	6	6	11	10	17	16 (94 %)

The staining results for the mAb clone JCB117 were influenced by the stainer platform: When the clone was used on, e.g., the Dako Autostainer platform, 36 out of 39 of the protocols (92%) gave a sufficient result (of which 30 were optimal - 77 %). In contrast, on the Ventana BenchMark instrument, only 17 out of 25 protocols (68%) gave a sufficient staining, of which 3 (12 %) resulted in an optimal staining, the latter all based on a high Ab concentration (1:25 – 1:100).

The Dako RTU format of the mAb clone JCB117 was very robust as all of 13 stains based on this IHC platform were assessed as sufficient (10 of them optimal). In contrast only 4 of 13 stains based on the same clone and the RTU system from Ventana were assessed as sufficient (and none were assessed as optimal). However, the RTU format of the rmAb clone SP18, Ventana gave an optimal result in all of 3 laboratories using the Ventana IHC platform.

This was the 3rd assessment of CD79a in NordiQC. A relatively constant proportion of sufficient results have been seen in the three runs (table 3).

Table 3. **Proportion of sufficient results for CD79a in the three NordiQC runs performed**

	Run 6 2002	Run 21 2007	Run 29 2010
Participants, n=	52	112	141
Sufficient results	83 %	78 %	75 %

Tonsil and appendix are reliable positive controls: The germinal centre B-cells must show at least a moderate and distinct membranous reaction. If only the mantle zone B-cells is distinctively demonstrated, the protocol is too insensitive to detect CD79a in neoplasias with a low CD79a expression such as plasmacytoma and Pre-B-ALL.

Conclusion

The mAb clone JCB117 and the rmAb SP18 are both recommendable Abs for the demonstration of CD79a. HIER is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated. Tonsil and appendix are appropriate controls: The germinal centre B-cells must show at least a moderate and distinct membranous staining reaction, while no staining is to be seen in the smooth muscle cells.

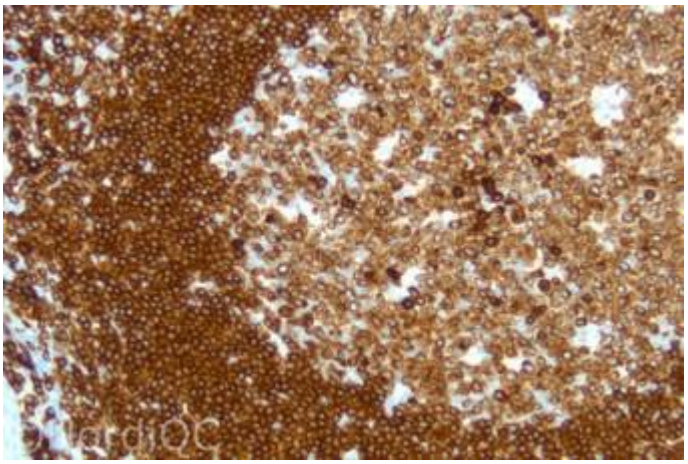


Fig. 1a
Optimal CD79a staining of the tonsil using the mAb clone SP18. The mantle zone B-cells show an intense membranous staining, while the germinal centre B-cells show a moderate staining. Plasma cells and late stage germinal centre B-cells show a strong cytoplasmic staining. Also compare with Figs. 2a & 3a – same protocol.

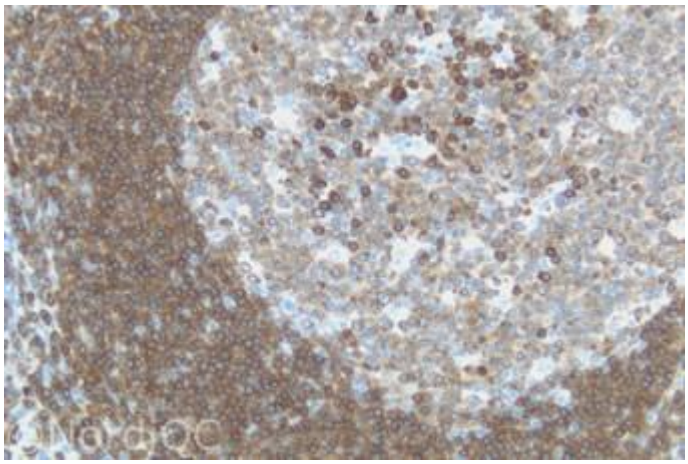


Fig. 1b
CD79a staining of the tonsil using an insufficient protocol based on the mAb clone JCB117 used too diluted – same field as in Fig. 1a.
The mantle zone B-cells and the late stage germinal centre B-cells are distinctively demonstrated, while the germinal centre B-cells show a weak and diffuse staining. Also compare with Figs. 2b & 3b – same protocol.

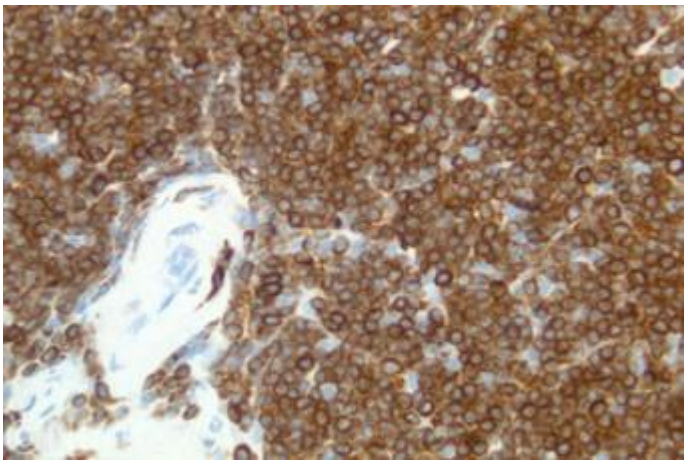


Fig. 2a
Optimal CD79a staining of the B-CLL using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct staining. No background reaction is seen.

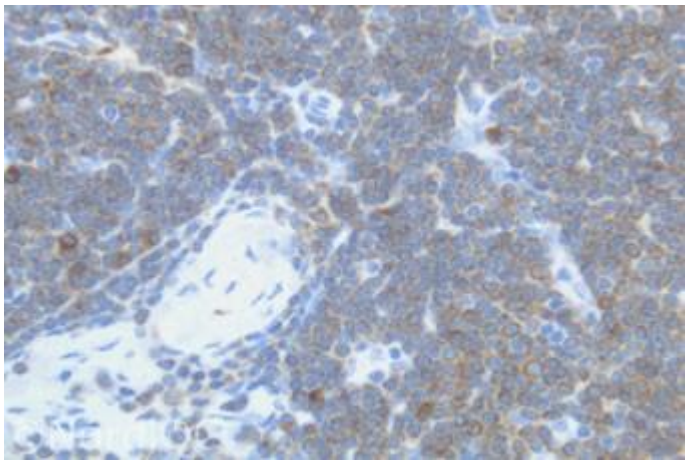


Fig. 2b
Insufficient CD79a staining of the B-CLL using same protocol as in Fig. 1b - same field as in Fig 2a. The neoplastic cells only show a weak or equivocal staining. Also compare with Fig. 3b – same protocol.

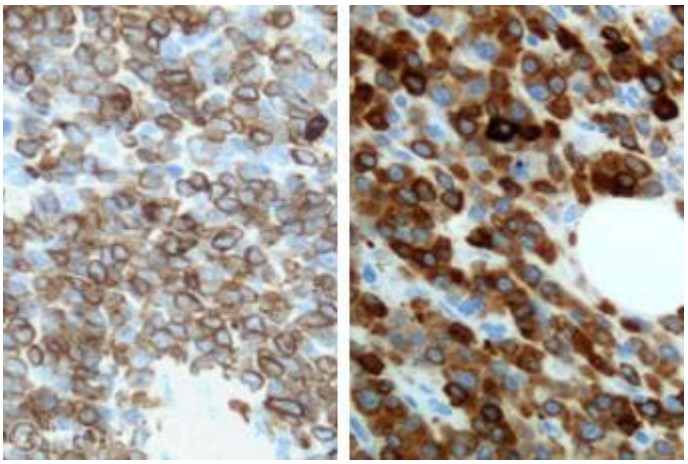


Fig. 3a
Optimal CD79a staining using same protocol as in Figs. 1a & 2a.
Left: Pre-B-ALL: The majority of the cells show a moderate and distinct predominantly membranous staining.
Right: Plasmacytoma: The majority of the neoplastic cells show a moderate cytoplasmic staining – entrapped normal plasma cells show a strong staining.

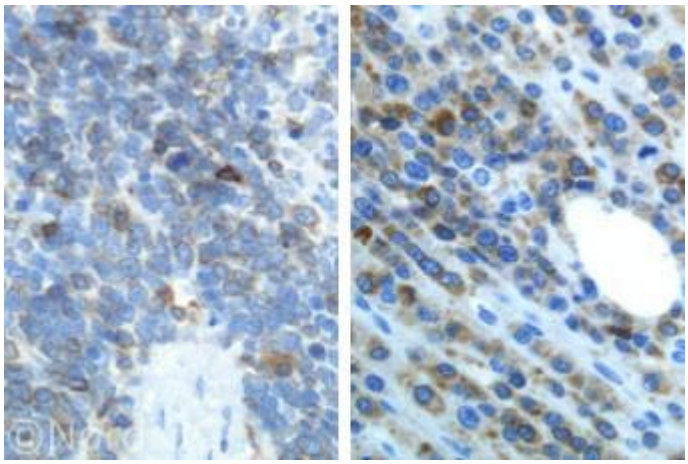


Fig. 3b
Insufficient CD79a staining using the same protocol as in Figs. 1b & 2b. – same fields as in Fig. 3a.
Left: Pre-B-ALL: Only scattered neoplastic cells show a weak and equivocal membranous staining.
Right: Plasmacytoma: Only scattered neoplastic cells show a weak and equivocal staining.

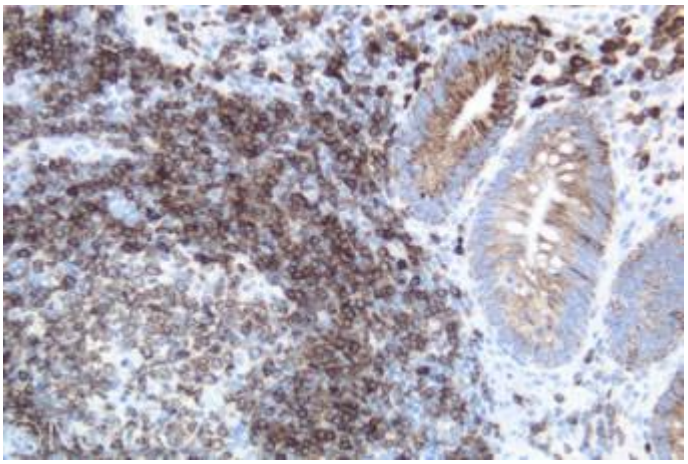


Fig. 4a
Insufficient CD79a staining of the appendix using the mAb clone HM57. The mantle zone B-cells and plasma cells in lamina propria show a strong and distinct reaction. However, also the epithelial cells show a cytoplasmic staining. All of 8 protocols based on clone HM57 were assessed as insufficient based on a reaction pattern as shown in Figs. 4a & 4b.

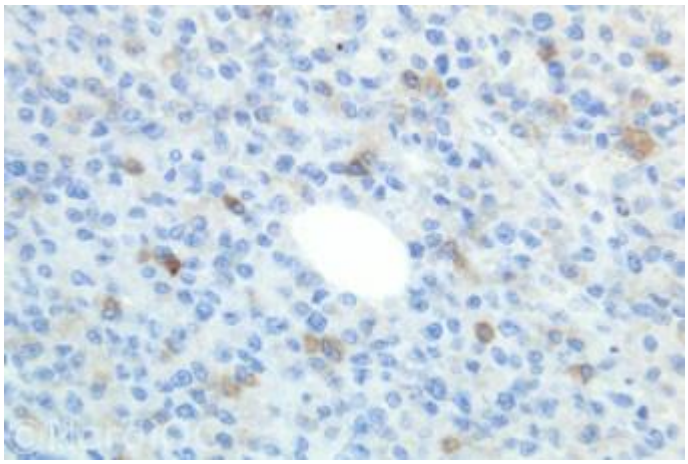


Fig. 4b
Insufficient staining of the plasmacytoma using same protocol as in Fig. 4a based on the mAb clone HM57.
Only scattered neoplastic cells show a weak and diffuse cytoplasmic staining. Compare with the optimal result in Fig. 3a right, same field.

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