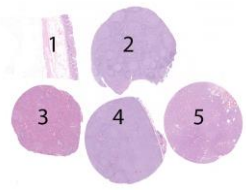
Assessment Run 45 2015
CD79a

Material
The slide to be stained for CD79a comprised:



1. Colon, 2. Tonsil, 3. Precursor B-ALL, 4. B-CLL, 5. Plasmacytoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing CD79a staining as optimal included:

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

Participation

Number of laboratories registered for CD79a, run 45	270
Number of laboratories returning slides	245 (91%)

Results
245 laboratories participated in this assessment. 194 (79%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful CD79a antibodies
- Too low concentration of the primary Ab
- Insufficient HIER - too short efficient HIER time

Performance history
This was the third NordiQC assessment of CD79. The pass rate of this run was similar to the two former runs in 2007 (Run 21) and 2010 (Run 29) as shown in table 2.

Table 2: **Proportion of sufficient results for CD79a in the three NordiQC runs performed**

	Run 21 2007	Run 29 2010	Run 45 2015
Participants, n=	112	141	245
Sufficient results	78%	75%	79%

Conclusion
The mAb clone **JCB17** and rmAb clone **SP18** are both recommendable antibodies for the demonstration of CD79a. The mAb clones 11D10, 11E3 and HM57 were all less successful and should not be used. Irrespective of the clone applied, efficient HIER, preferable in an alkaline buffer, and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. Compared to concentrated formats within laboratory developed assays, the Ready-To-Use systems from Dako and Ventana provided the highest proportion of sufficient and optimal results.
Tonsil and colon is recommended as positive and negative tissue control: Virtually all mantle zone B-cells must show a strong and distinct membranous staining reaction, while an at least moderate staining reaction of the germinal centre B-cells must be seen. Plasma cells must show a moderate to strong cytoplasmic staining reaction, while no staining of epithelial cells should be seen.

Table 1. **Antibodies and assessment marks for CD79a, run 45**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **11D10**	1	Leica/Novocastra	0	0	0	1	-	-
mAb clone **11E3**	3	Leica/Novocastra	0	0	0	3	-	-
mAb clone **HM57**	2	Dako	0	0	0	2	-	-
mAb clone **JCB117**	94 3	Dako Thermo/NeoMarkers	37	35	19	6	74%	74%
rmAb clone **SP18**	12 3 2 1 1	Thermo/NeoMarkers Spring Bioscience Cell Marque Nordic Biosite Zytomed	4	14	0	1	95%	83%
Ready-To-Use antibodies								
mAb clone **11E3 PA0192**	6	Leica/Novocastra	0	0	3	3	-	-
mAb clone **HM46/A9 PM067**	1	Biocarea	0	0	0	1	-	-
mAb clone **JCB117 IR/IS621**	40	Dako	23	11	5	1	85%	89%
mAb **JCB117 GA621**	11	Dako	9	2	0	0	100%	100%
mAb **JCB117 760-2639***	2	Ventana/Cell Marque	0	1	1	0	-	-
mAb clone **JCB117 PA0599**	1	Leica/Novocastra	0	0	0	1	-	-
rmAb clone **SP18 790-4432**	58	Ventana	50	6	0	2	97%	96%
rmAb clone **SP18 MAD-00032QD**	2	Master Diagnostica	0	0	2	0	-	-
rmAb clone **SP18 179R-18**	1	Cell Marque	0	1	0	0	-	-
rmAb clone **SP18 RMA-0552**	1	Maixin	1	0	0	0	-	-
Total	245		124	70	30	21	-	
Proportion			51%	28%	12%	9%	79%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.
* Discontinued product.

Detailed analysis of CD79a, Run 45
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **JCB117**: Protocols with optimal results were typically based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (6/16)*, TRS pH 9 (Dako) (3/7), Cell Conditioning 1 (CC1, Ventana) (11/44), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (7/10), BERS1 (Leica) (2/2), Tris-EDTA pH 9 (7/9) or Citrate pH 6 (1/6) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:600. Using these protocol settings, 67 of 90 (74%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **SP18**: Protocols with optimal results were based on HIER using CC1 (Ventana) (4/8) as retrieval buffer. The mAb was diluted in the range of 1:300-1:500. Using these protocol settings, 5 of 6 (83%) laboratories produced a sufficient staining result.

Table 3: **Proportion of optimal results for CD79a for the two most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **JCB117**	9/16** (56%)	0/1	11/31 (36%)	-	6/8 (75%)	2/2
rmAb clone **SP18**	0/2	-	4/6 (67%)	-	0/2	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer).

Ready-To-Use antibodies and corresponding systems
mAb clone **JCB117**, product no. **IS621/IR621**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min. at 95-99°C), 15-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 34 of 38 (89%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **JBC117**, product no. **GA621**, Dako, Dako Omnis:
Protocols with optimal results were based on HIER using TRS pH 9 (efficient heating time 24-30 min. at 97°C), 20-30 min. incubation of the primary Ab and EnvisionFlex/FLEX+ (GV800/GV800+GV821) as detection system. Using these protocol settings, 10 of 10 (100%) laboratories produced a sufficient staining result.

rmAb clone **SP18**, product no. **790-4432**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal result were typically based on HIER using Cell Conditioning 1 (efficient heating time 30-90 min.) and 16-56 min. incubation of the primary Ab. UltraView (760-500) or OptiView (760-700) were used as detection systems. Using these protocol settings 54 of 56, (96%) laboratories produced a sufficient staining result.

rmAb clone **SP18**, product no. **RMA-0552**, Maixin, manual staining:
One protocol with an optimal result was based on HIER using Citrate buffer pH 6 (efficient heating time 2 min. at 120°C), 60 min. incubation of the primary Ab. and KIT-5230 (Maixin) as detection system.

Comments
In concordance with the previous NordiQC assessments of CD79a, the prevalent feature of an insufficient staining result was an overall too weak or a false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 88% of the insufficient results (45 of 51). The majority of the laboratories were able to demonstrate CD79a in high level antigen expressing structures such as mantle zone B-cells, plasma cells and the neoplastic cells of the B-CLL, whereas the demonstration of CD79a in the neoplastic cells of the pre-B-ALL and plasmacytoma was more challenging and required an optimally calibrated protocol.

mAb clone JCB117 was the most widely used antibody for the demonstration of CD79a. Used as a concentrate within a laboratory developed (LD) assay, it provided an optimal staining result on all the three main IHC platforms from Dako, Leica and Ventana. However, as observed in the previous assessment of CD79a, run 29 2010, with a slightly lower pass rate on the BenchMark platform compared to the other platforms.
Efficient HIER, preferable in an alkaline buffer and a careful calibration of the primary Ab concentration were the main protocol prerequisites for an optimal staining result, whereas the application of a 3-step (versus a 2-step) detection system was of less importance. Using HIER in an alkaline buffer in combination with a 2-step detection system as EnVision FLEX (Dako), mAb JCB117 typically was applied in the range of 1:25-100 to obtain an optimal staining result, and 1:100-600 using a 3-step system as EnVision FLEX+ (Dako) or Refine (Leica).

The rmAb clone SP18 also provided optimal results within a LD assay. Optimal results were only observed on the BenchMark platform (Ventana) using a titre of the primary Ab in the range of 1:300-500, HIER in CC1 for 32-48 min. and a 3-step multimer based detection system as OptiView. Using similar protocol settings on e.g. Bond, Leica or Autostainer Link, Dako an aberrant staining reaction in epithelial cells of the colon typically was seen. No specific cause for the aberrant staining reaction as e.g. lot. no. of Ab or Ab provider could be identified.

In this assessment, the corresponding RTU systems for the mAb clone JCB117, Dako (GA/IR/IS649) and rmAb clone SP18, Ventana (790-4432) respectively, provided a higher pass rate and proportion of optimal results compared to LD assays using same clones as concentrate (see table 1).
Optimal results for these two RTU products were typically obtained using the official protocol recommendations given by the companies. Laboratory modified protocol settings (typically adjusting HIER, incubation time of the primary Ab and/or choice of detection system) could also provide sufficient and optimal result.

The mAb clones 11D10 and 11E3 consistently, both as a concentrate within a LD assay and as RTU format, provided an insufficient staining result. Plasma cells and mantle zone B-cells with high level CD79a expression typically were identified but no reliable staining reaction in the neoplastic cells of the pre-B-ALL and plasmacytoma could be identified.
The mAb clone HM57 was observed both to give a reduced specific staining reaction in both normal and neoplastic B-cells and simultaneously also provided an aberrant staining reaction of epithelial cells and smooth muscle cells.

Controls
Tonsil and colon (or appendix) is recommended as positive and negative tissue controls for CD79a. In tonsil, the protocol must be calibrated to provide a distinct and strong membranous staining reaction in all mantle zone B-cells. Virtually all germinal centre B-cells must at least display a moderate and distinct staining reaction. No staining reaction must be seen in T-cells and squamous epithelial cells.
In colon (or appendix), plasma cells in lamina propria must show a moderate to strong cytoplasmic staining reaction. No staining reaction should be seen in the epithelial cells.

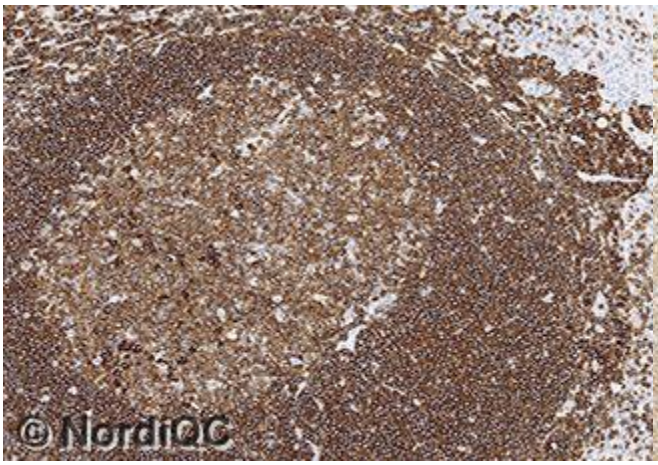


Fig. 1a
Optimal CD79a staining of the tonsil using the mAb clone JCB117 as Ready-To-Use format (GA621, Dako), with HIER in TRS High pH 9 for 30 min., a 3-step polymer based detection kit and performed on Omnis, Dako.
Mantle zone B-cells show an intense membranous staining reaction, while the germinal centre B-cells show a moderate staining reaction. Plasma cells and late stage germinal centre B-cells show a strong cytoplasmic staining reaction.
Also compare with Figs. 2a – 5a, same protocol.

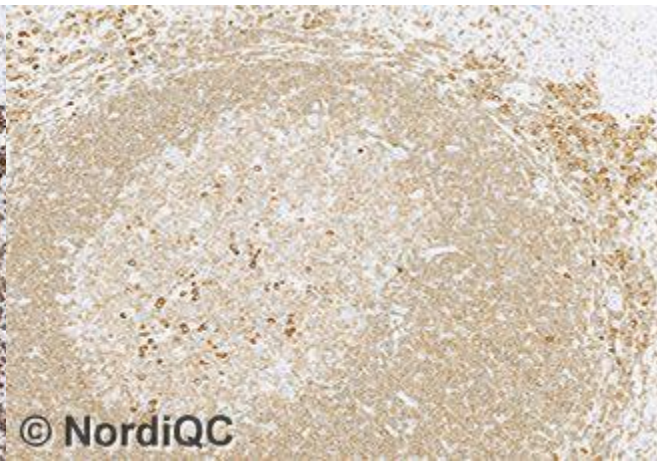


Fig. 1b
CD79a staining of the tonsil using the mAb clone JCB117 with an insufficient protocol – same field as in Fig. 1a.
The primary Ab was used at a titre of 1:500 and a 2-step multimer based detection system providing a too low sensitivity.
The mantle zone B-cells and the late stage germinal centre B-cells are demonstrated, while the germinal centre B-cells only show a weak and diffuse staining reaction.
Also compare with Figs. 2b & 3b – same protocol.

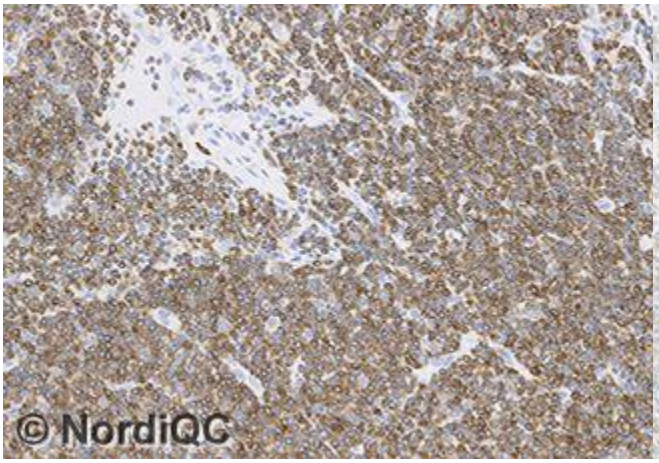


Fig. 2a
Optimal CD79a staining of the B-CLL using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a moderate and distinct membranous staining reaction.
No background reaction is seen.

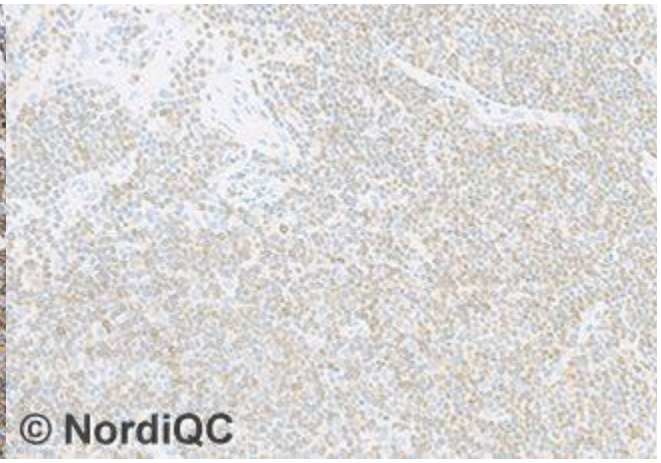


Fig. 2b
Insufficient CD79a staining of the B-CLL using same protocol as in Fig. 1b - same field as in Fig. 2a.
The neoplastic cells only show a weak and equivocal staining reaction.
Also compare with Fig. 3b – same protocol.

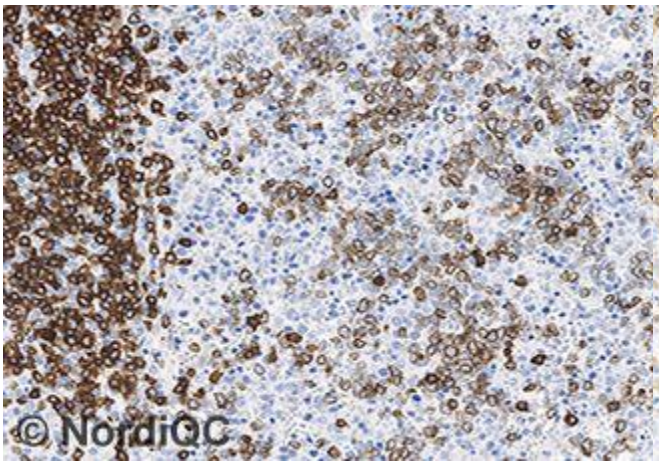


Fig. 3a
Optimal CD79a staining of the Precursor-B-ALL using same protocol as in Figs. 1a and 2a.
The majority of neoplastic cells show a weak to strong staining reaction.
At left, remnants of normal B-cells show a strong staining reaction.

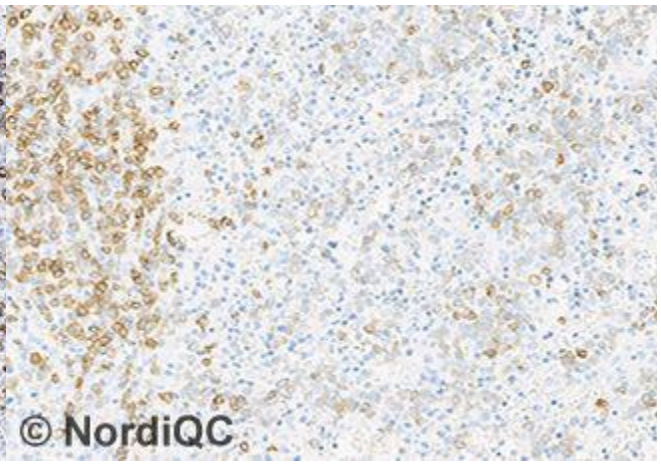


Fig. 3b
Insufficient CD79a staining of the Precursor-B-ALL using the same protocol as in Figs. 1b & 2b - same field as in Fig. 3a.
The intensity and proportion of neoplastic cells is significant reduced compared to the level expected and obtained by optimal protocol settings and only remnants of normal B-cells are distinctively demonstrated.

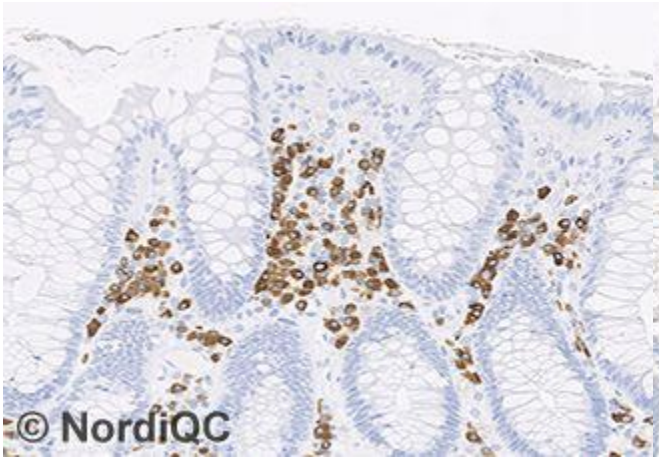


Fig. 4a
Optimal CD79a staining of colon using same protocol as in Figs. 1a - 3a.
Plasma cells show a moderate to strong cytoplasmic staining reaction.
No background reaction is seen.

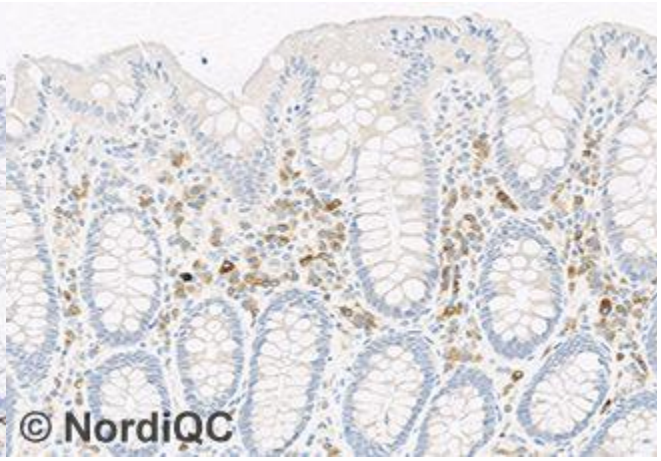


Fig. 4b
CD79a staining of the colon using an insufficient protocol based on the mAb clone 11E3.
The intensity and proportion of plasma cells demonstrated is reduced compared to the level expected.
However also compare with Fig. 5b – same protocol

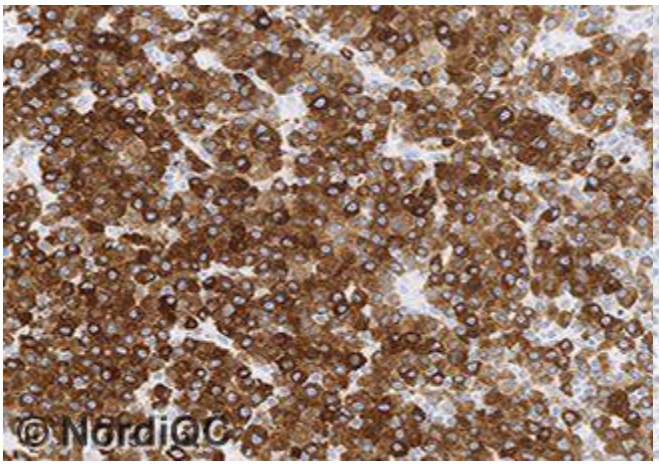


Fig. 5a
Optimal CD79a staining of the plasmacytoma using same protocol as in Figs. 1a - 4a.
Virtually all neoplastic cells show a moderate cytoplasmic staining reaction.

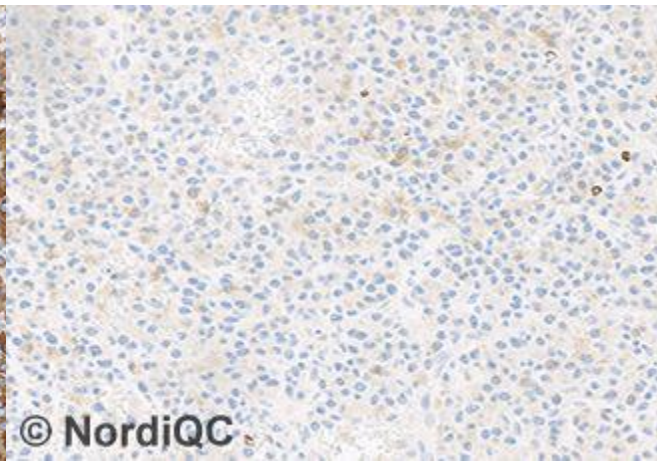


Fig. 5b
Insufficient CD79a staining of the plasmacytoma using same protocol as in Fig. 4b.
Only scattered normal B-cells are demonstrated, while the neoplastic cells are negative.
9 of 9 protocols based on mAb clone 11E3 provided an insufficient result due to a too weak or completely false negative staining reaction in both the plasmacytoma and the precursor B-ALL.

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