Assessment Run 63 2021
CD79a

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD79a, identifying B-cell neoplasm in the diagnostic work up of both lymphomas and leukamias. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD79a (see below).

Material
1. Tonsil, 2. Appendix, 3. Precursor B-ALL (pre-B-ALL), 4-5. Diffuse Large B-Cell Lymphomas (DLBCL)

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 appendix.
- A strong, predominantly cytoplasmic staining reaction of plasma cells and the late stage activated germinal centre B-cells in the tonsil and appendix.
- An at least moderate membranous staining reaction of all neoplastic cells in the pre-B-ALL.
- A strong, predominantly membranous staining reaction of all neoplastic cells in the DLBCL (tissue core no. 4).
- An at least moderate, predominantly membranous staining reaction of virtually all neoplastic cells in the DLBCL (tissue core no. 5).
- No staining of other cells including T-cells, epithelial and smooth muscle cells of the appendix.

Participation

Number of laboratories registered for CD79a, run 63	349
Number of laboratories returning slides	324 (93%)

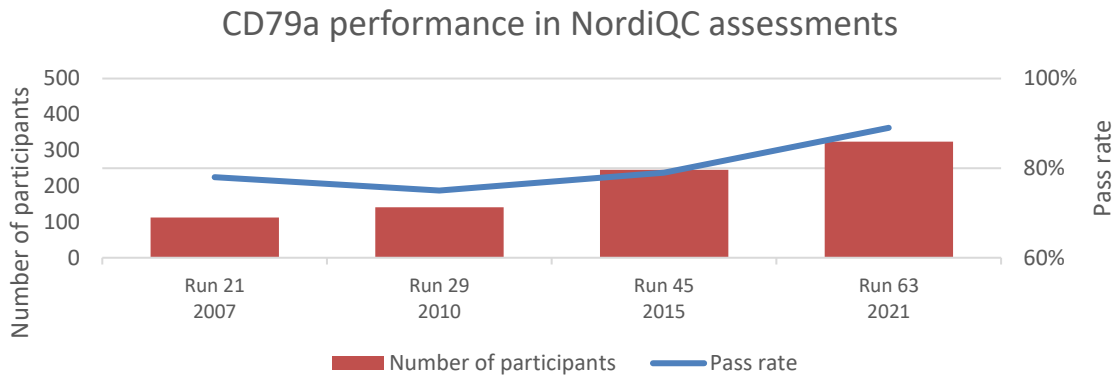
Results
At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

324 laboratories participated in this assessment and 89% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Too diluted primary Ab.
- Use of less sensitive detection systems.
- Less successful primary antibodies

Performance history
This was the fourth NordiQC assessment of CD79a. The pass rate increased significantly compared to the previous runs (see Graph 1).

Graph 1. **Proportion of sufficient results for CD79a in the four NordiQC runs performed**



Conclusion

The widely used mAb clone **JCB117** and rmAb clone **SP18** are both recommendable antibodies for the demonstration of CD79a. Optimal results could also be obtained using the mAb clones **GM101, C4C4** and the rmAb clones **BSR20, GR019 and ZR237**. Irrespective of the clone applied, efficient HIER, preferable in an alkaline buffer, and careful calibration of the primary antibody and use of a 3-step multimer/polymer detection system were the most important requirements for an optimal staining result. The Ready-To-Use (RTU) systems GA621 (Dako/Agilent), PA0599 (Leica Biosystems), both based on the mAb clone JCB117 and 790-4432 (Roche/Ventana) based on the rmAb clone SP18 provided superior performance.

Tonsil and colon/appendix are 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 strong cytoplasmic staining reaction, while no staining of epithelial cells should be seen.

Table 1. **Antibodies and assessment marks for CD79a, Run 63**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **JCB117**	77 4 4 1 1	Dako/Agilent Cell Marque Leica Biosystems Zytomed Systems Scytek	37	34	11	5	82%	43%
mAb clone **HM47/A9**	1 1 1	Thermo Scientific Biocare Medica Diagnostic Biosystems	0	1	0	2	-	-
mAb clone **11E3***	1	Leica Biosystems	0	0	0	1	-	-
mAb clone **HM57***	1	Dako/Agilent	0	0	0	1	-	-
rmAb clone **SP18**	4 3 1 1 1	Thermo Scientific Cell Marque BioGenex Spring Bioscience Epredia	8	2	0	0	100%	80%
rmAb clone **BSR20**	1	Nordic Biosite	1	0	0	0		
rmAb clone **ZR237**	1	Zeta Corp.	1	0	0	0		
rmAb clone **BP6040**	1	Biolynx Biotechnology	0	0	1	0		
rmAb clone **Unknown**	1	Quartett	1	0	0	0		
Ready-To-Use antibodies								
mAb clone **JCB117, IR621[3]**	12	Dako/Agilent	4	6	2	0	83%	33%
mAb clone **JCB117, IR621[4]**	15	Dako/Agilent	9	4	2	0	87%	60%
mAb clone **JCB117, GA621[3]**	37	Dako/Agilent	32	5	0	0	100%	86%
mAb clone **JCB117, GA621[4]**	21	Dako/Agilent	13	4	4	0	81%	62%
mAb clone **JCB117, PA0599[3]**	9	Leica Biosystems	8	1	0	0	100%	89%
mAb clone **JCB117, PA0599[4]**	8	Leica Biosystems	7	0	1	0	88%	88%
mAb clone **GM101, GT2325**	1	GeneTech	1	0	0	0	-	-
mAb clone **C4C4, CCM-0734**	1	Celnovte Biotechnology	1	0	0	0	-	-
mAb clone **HM47/A9 PDM125**	1	Diagnostic Biosystems	0	0	0	1	-	-
rmAb clone **GR019, 8278-C010**	3	Sakura FineTek	1	0	2	0	-	-
rmAb clone **SP18, 790-4432[3]**	8	Ventana/Roche	5	3	0	0	100%	63%
rmAb clone **SP18, 790-4432[4]**	92	Ventana/Roche	77	13	1	1	98%	84%
rmAb clone **SP18, 179R-17/18**	6	Cell Marque	4	2	0	0	100%	67%
rmAb clone **SP18, MAD-000320QD**	3	Master Diagnostica	1	2	0	0	-	-
rmAb clone **SP18, AN767**	1	BioGenex	0	1	0	0	-	-
Total	324		211	78	24	11	-	
Proportion			65%	24%	8%	3%	89%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).
* Discontinued

Detailed analysis of CD79a, Run 63
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **JCB117**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (7/15)*, TRS High pH (Dako/Agilent) (1/1), Cell Conditioning 1 (CC1, Ventana/Roche) (11/41), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (15/18) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (3/5) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200. Using these protocol settings, 56 of 70 (80%) 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 all based on HIER in an alkaline buffer using TRS pH 9 (3-in-1) (2/2), CC1 (4/6) or BERS2 (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:200-1:800. Using these protocol settings, 9 of 9 (100%) laboratories produced a sufficient staining result.

rmAb clone **BSR20**: One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 as retrieval buffer. The mAb was diluted 1:100 and ZytoMed Detection Kit (ZUC032-100, ZytoMed) was used as the detection system.

rmAb clone **ZR237**: One protocol with an optimal result was based on HIER using BERS2 as retrieval buffer. The mAb was diluted 1:100 and Zeta Universal HRP Polymer (ZD10, Zeta Corporation) was used as the detection system.

Table 2. **Proportion of optimal results for CD79a for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark GX / XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **JCB117**	5/8** (63%)	0/1	2/7 (29%)	-	10/39 (26%)	0/1	10/11 (91%)	3/5 (60%)
rmAb clone **SP18**	-	-	2/2	-	4/5 (80%)	-	2/2	-

* 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. **IR621**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8002+K8021) as detection systems. Using these protocol settings, 15 of 17 (88%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **JC117**, product no. **GA621**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 30 min. at 97°C), 20-30 min. incubation of the primary Ab and Envision FLEX+ (GV800/GV821) as detection system. Using these protocol settings, 39 of 40 (98%) laboratories produced a sufficient staining result.

mAb clone **JCB117** product no. **PA0599**, Leica Biosystems, Bond III/MAX:
Protocols with optimal results were typically based on HIER using either Bond Epitope Retrieval Solution 1 (BERS1) or BERS2 (efficient heating time 20 min. at 99-100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 13 of 14 (93%) laboratories produced a sufficient staining result.

mAb clone **C4C4**, product no. **CCM-0734**, Celnovte Biotechnology, CNT330 Stainer:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time 20 min. at 100°C), 30 min. incubation of the primary Ab and CNTVision Super PolyDetection Kit (SD5100) as detection system.

mAb clone **GM101**, product no. **GT2325**, Gene Tech, Gene Tech Genestainer:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time 20 min. at 95°C), 50 min. incubation of the primary Ab and GTVision Detection Kit (GK8007) as detection system.

rmAb clone **SP18**, product no. **790-4432**, Ventana/Roche, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C) and 16-32 min. incubation of the primary Ab. UltraView (760-500) with amplification (760-080) or OptiView (760-700) were used as detection systems. Using these protocol settings, 83 of 85 (98%) laboratories produced a sufficient staining result.

rmAb clone **GR019**, product no. **8278-C010**, Sakura FineTek, Tissue-Tek Genie Advanced:
One protocol with an optimal result was based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (efficient heating time 60 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit (8826-K250) as detection system.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for CD79a for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb JCB117 **IR621**	83% (10/12)	33% (4/12)	100% (10/10)	80% (8/10)
Dako Omnis mAb JCB117 **GA621**	100% (37/37)	86% (32/37)	80% (16/20)	60% (12/20)
Leica Bond III/MAX mAb JCB117 **PA0599**	100% (9/9)	89% (8/9)	88% (7/8)	88% (7/8)
VMS Ultra/XT/GX rmAb SP18 **790-4432**	100% (8/8)	63% (5/8)	98% (90/92)	84% (77/92)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In this assessment and in concordance with the previous NordiQC runs for CD79a, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells expected to be demonstrated. This staining pattern was seen in 86% (30/35) of the insufficient results. In the remaining insufficient results, a combination of different reaction patterns was seen e.g., both false positive and false negative staining result simultaneously. Virtually all 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 DLBCL (tissue core no. 4), whereas the demonstration of CD79a in the neoplastic cells of the pre-B-ALL and the DLBCL (tissue core no. 5) was more challenging and required an optimally calibrated protocol.

The mAb clone JCB117 and the rmAb clone SP18 were the most widely used antibodies for demonstration of CD79a and applied by 95% (309/324) of the laboratories (see Table 1).
Used as concentrated format within laboratory developed (LD) assays, the mAb clone JCB117 provided 82% (71/87) sufficient results of which 43% (37/87) were assessed as optimal. As shown in Table 2, assays based on the mAb clone JCB117 gave optimal results on the four main automated platforms. The most prevalent cause for an insufficient staining result was related to use of a less sensitive detection system. Using optimal protocol settings, efficient HIER in an alkaline buffer and applying a 2-step polymer/multimer based detection system (e.g., Envision Flex or UltraView), only 57% (16/28) of the protocols produced a sufficient staining result of which 18% (5/28) were assessed as optimal. In comparison, using the exact same protocol settings except for substituting the 2-step polymer/multimer with a 3-step detection system (e.g., OptiView, Bond Refine or Envision Flex+), 100% (33/33) of the results were assessed as sufficient and 58% (19/33) were giving an optimal mark. In addition, the proportion of optimal results was highly influenced by which platform the staining was accomplished on (see Table 2). The performance of mAb clone JCB117 was found superior on Dako Autostainer and Bond compared to Omnis and Benchmark. For the vast majority of protocols applied on the Omnis or the BenchMark, high analytical sensitive protocol settings were required for optimal performance e.g., use of HIER in alkaline buffer (TRS pH 9 or CC1), high concentration of the primary ab (dilution range 1:25-1:100) and the use of a 3-step multimer/polymer detection system (OptiView or Envision Flex+).
For Bond Max/III users applying the mAb clone JCB117 within a LD-assay, it was observed that optimal results could be obtained by HIER in both low and high pH buffers. However, if HIER was performed in an acidic buffer (BERS1), the concentration of the primary Ab needs to be increased (approximately 4-fold, e.g., from 1:200 to 1:50) compared to the use of an alkaline HIER buffer. Thus, the choice of HIER buffer

on the Bond platform seems less important as long as the titer of the primary antibody is calibrated correctly, providing the expected level of analytical sensitivity fulfilling intended use of the test.

Ten participants used the rmAb clone SP18 within a LD-assay and the Ab was very robust providing a pass rate of 100%. All protocols were based on HIER in an alkaline buffer and optimal results could be obtained on the three main fully automated systems BenchMark Ultra, Bond III and Omnis (see Table 2). Both high and low analytical sensitive protocol settings could be applied for optimal performance e.g., use of 2- and 3-step polymer/multimer based detection systems.

In total, 67% (218/324) of the laboratories used a RTU format for demonstration of CD79a and almost all (97%, 212/218) were based on the mAb clone JCB117 or the rmAb clone SP18. As shown in Table 3, and applying vendor recommended protocol settings (VRPS), the RTU systems GA621 (Dako/Agilent), PA0599 (Leica), both based on the mAb clone JCB117, and 790-4432 (Roche/Ventana) based on the rmAb clone SP18 provided superior performance. Grouped together, these three RTU systems provided 100% (54/54) sufficient results. However, it was observed that the proportion of optimal results was significantly lower using the RTU 790-4432 based on SP18 compared to the RTU systems GA621 and PA0599 based on JCB117 and mainly being related to the vendor recommendation to the detection systems. For the RTU system 790-4432, the 2-step multimer detection system UltraView is recommended by the vendor, whereas for GA621 and PA0599, the recommendation is to use a 3-step polymer based detection system, Envision Flex+ and Bond Refine, respectively. Laboratory modified protocol settings (LMPS) could also provide a high proportion of sufficient and optimal results (see Table 3), and for the RTU system 790-4432, a substantial number of laboratories modified the basic protocol settings, resulting in a significant increase of optimal results compared to applying VRPS. This improvement was primarily related to the use of a 3-step multimer based detection system, and it was observed that for laboratories substituting UltraView with Optiview (with or without amplification) the proportion of sufficient results was 100% (48/48), as such concordant to the level seen for laboratories using VRPS (see Table 3), but the proportion of optimal results increased considerably to 94% (45/48) (compared to only 63% with the VRPS, see Table 1). The same pattern was seen with the RTU system IR621 based on the mAb clone JCB117 (Autostainer), and by substituting the vendor recommended 2-step detection system Envision Flex with the 3-step system Envision Flex+, the proportion of sufficient and optimal results increased significantly (see Table 3). Thus, the choice of detection system impacts the performance of the RTU assays and companies are obligated to continually up-date package inserts for a given RTU product to guide and provide the customers with best practices and "true plug-and play" protocols.

As described in the previous reports, the mAb clones HM57 and 11E1 are less successful antibodies for demonstration of CD79a, both providing poor performance. In addition, the mAb clone HM47/A9 also seems challenging and grouped together, only one protocol (1/6) was assessed as sufficient (giving the mark good). In total, these less successful antibodies accounted for 14% (5/35) of all insufficient results and cannot be recommended to use for diagnostics.

This was the fourth NordiQC assessment of CD79a and the pass rate increased significantly compared to the previous runs (see Graph 1). Virtually all assays (95%), both RTU formats and concentrates, were either based on the mAb clone JCB117 or the rmAb clone SP18. The prevalent requirements of an optimal result was use of HIER, preferable in an alkaline buffer, in combination with use of a sensitive 3-step detection system. Using these fundamental parameters, 99% (165/167) produced a sufficient result of which 81% (135/167) were optimal.
Importantly, protocols must stain according to the expected antigen levels, and both tonsil and appendix are essential and critical assay performance controls assisting to monitor the required level of the analytical sensitivity and specificity of the assay (see below).

Controls
Tonsil and colon/appendix are 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 membranous staining reaction. Plasma cells and late stage activated B-cells must show a strong cytoplasmic staining reaction. No staining reaction must be seen in T-cells and squamous epithelial cells. In colon/appendix, plasma cells in lamina propria mucosa must show a strong cytoplasmic staining reaction. No staining reaction should be seen in the epithelial cells.

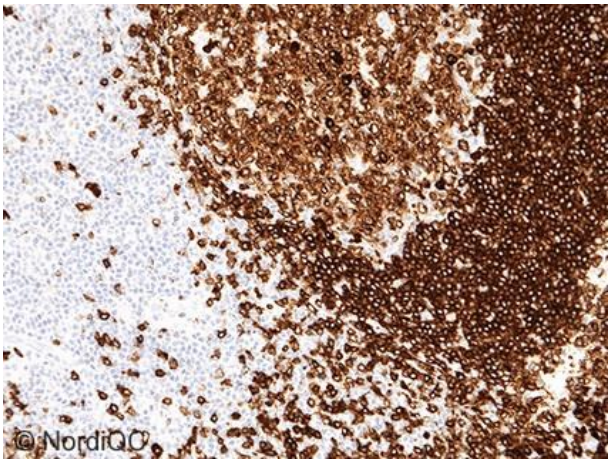


Fig. 1a (x200)
Optimal staining for CD79a of the tonsil using the mAb clone JCB117 as concentrate diluted 1:100, efficient HIER in TRS pH 9 and Envision Flex+ as the detection system on the Omnis platform (Dako/Agilent) - same protocol used in Figs. 2a - 5a. Virtually all mantle zone B-cells show a strong staining intensity, whereas intra germinal centre B-cells display a moderate to strong predominantly membranous staining reaction. Plasma cells and late stage activated B-cells show a strong cytoplasmic staining reaction.

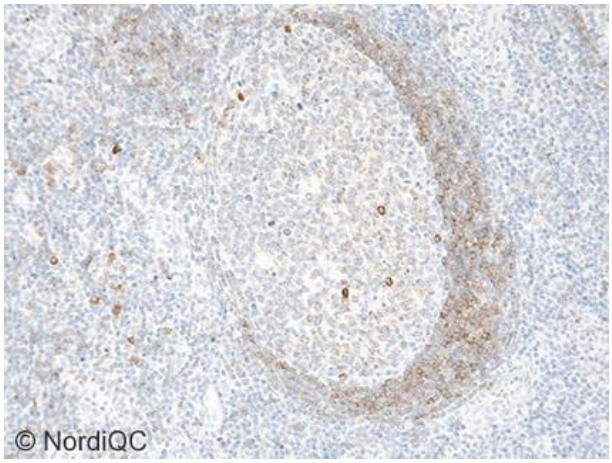


Fig. 1b (x200)
Insufficient staining for CD79a of the tonsil using the mAb clone JCB117 within a LD-assay on the Omnis platform (Dako/Agilent) with a
protocol providing too low analytic sensitivity - too diluted primary Ab (1:200) in combination with the less sensitive Envision Flex as detection system - same protocol used in Figs. 2b – 5b. The mantle zone B-cells are only weakly demonstrated and intra germinal centre B-cells are false negative – compare with Fig. 1a.

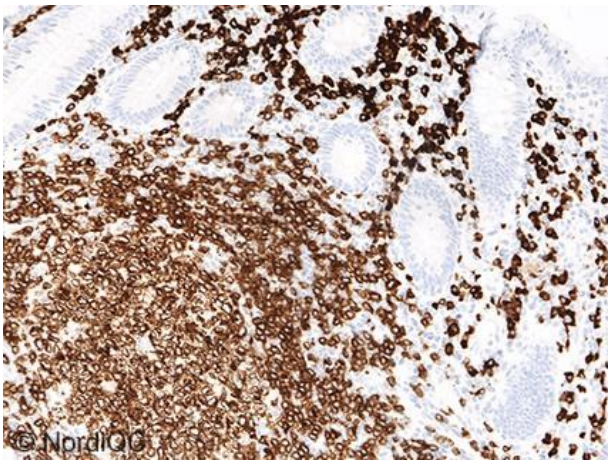


Fig. 2a (x200)
Optimal CD79a staining of the appendix using same protocol as in Fig. 1a. Virtually all B-cells cells show a strong, predominantly membranous staining reaction and plasma cells situated in lamina propria mucosa display a strong cytoplasmic reaction. Importantly, T-cells and epithelial cells are negative.

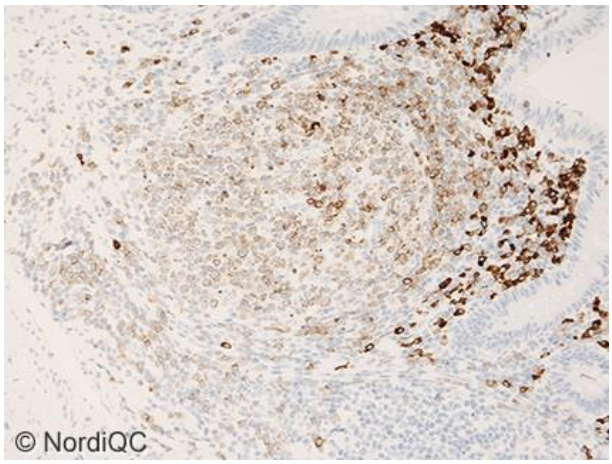


Fig. 2b (x200)
Insufficient CD79a staining of the appendix using same protocol as in Fig. 1b. The proportion and staining intensity of both B- and plasma cells is significantly reduced – compare with in Fig. 2a. The insufficient staining pattern obtained in both the tonsil and in the appendix impacts the interpretation of clinical specimens risking misdiagnosis - compare Fig. 3a-3b and 5a-5b.

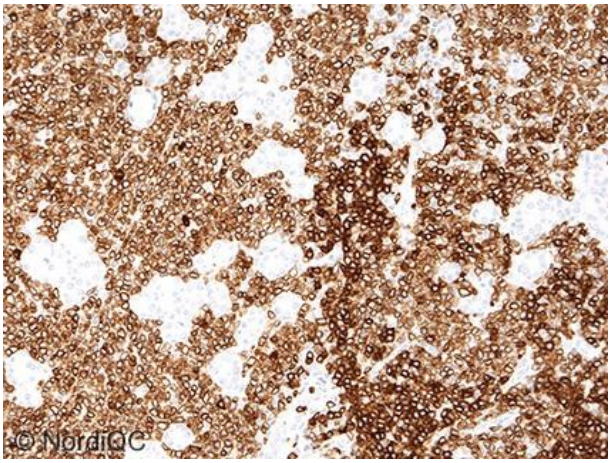


Fig. 3a (x200)
Optimal CD79a staining of the pre-B-ALL using same protocol as in Figs. 1a and 2a. All neoplastic B cells display a moderate and distinct membranous staining reaction, whereas normal B-cells intermingling between the neoplastic cells are strongly labelled. The epithelial cells of the salivary gland are as expected negative.

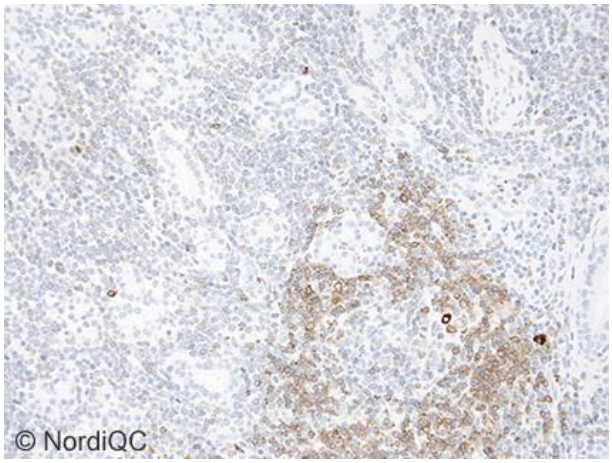


Fig. 3b (x200)
Insufficient CD79a staining of the pre-B-ALL using the same protocol as in Figs. 1b and 2b. The neoplastic B-cells are false negative and only normal B-cells with high level CD79a expression are weakly demonstrated. The normal B-cells is unreliable as internal positive tissue control and thus, laboratories should include relevant critical assay performance controls. Both tonsillar and appendiceal tissue (extern controls) are mandatory for evaluation of the required analytical sensitivity and specificity of the test – see criteria for assessing CD79a as optimal in the description above - compare with Fig. 3a.

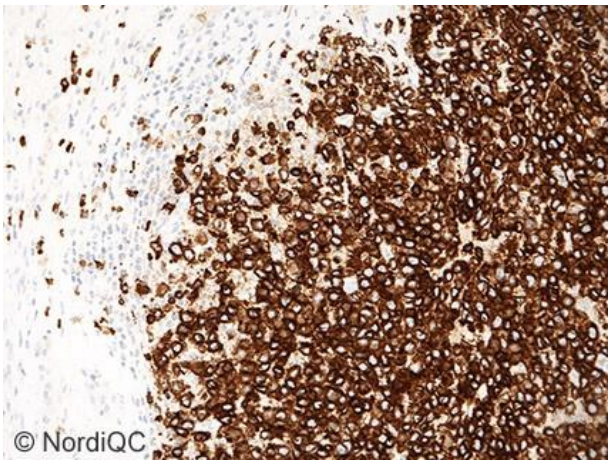


Fig. 4a (x200)
Optimal CD79a staining of the DLBCL (tissue core no. 4) using same protocol as in Figs. 1a - 3a. All neoplastic cells display a strong, predominantly membranous staining reaction.

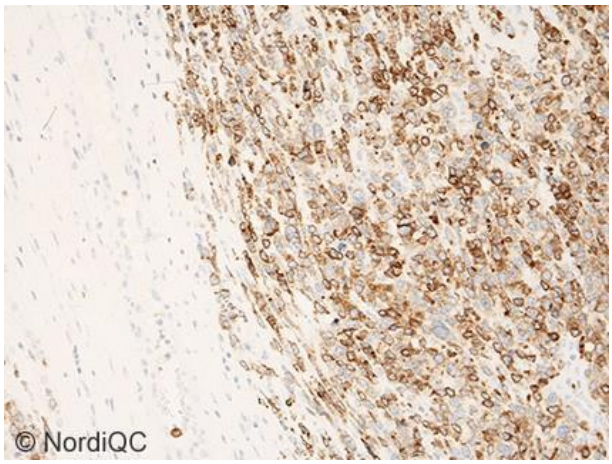


Fig. 4b (x200)
CD79a staining of the DLBCL (tissue core no. 4) using the same insufficient protocol as in Figs. 1b - 5b. Although the vast majority of the neoplastic cells display a weak to moderate staining intensity, the protocol provided an overall too low analytical sensitivity (see Figs. 1b-5b).

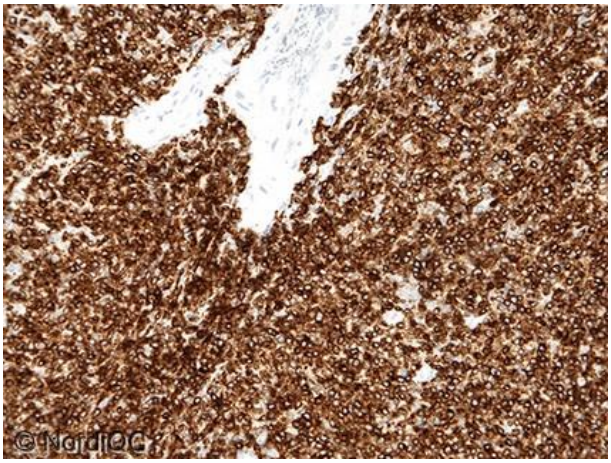


Fig. 5a (x200)
Optimal CD79a staining of the DLBCL (tissue core no. 5), using same protocol as in Figs. 1a - 4a. All the neoplastic B-cells show a strong, predominantly membranous staining reaction.

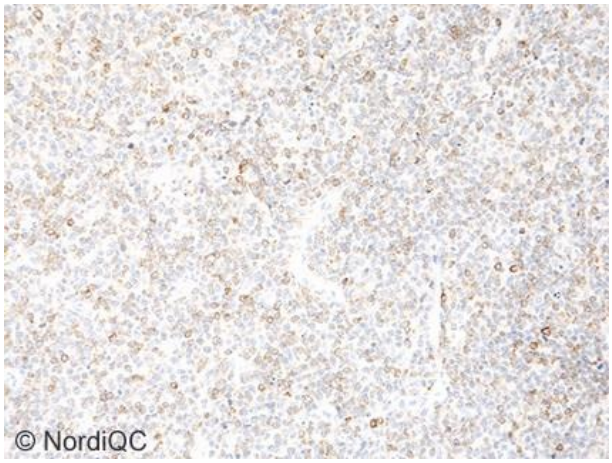


Fig. 5b (x200)
Insufficient CD79a staining of the DLBCL (tissue core no. 5), using same protocol as in Figs. 1b - 4b. The neoplastic B-cells are false negative or only faintly demonstrated – compare with Fig. 5a.

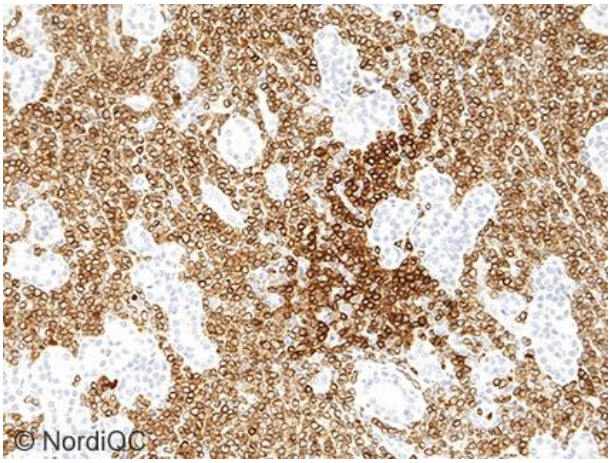


Fig. 6a (x200)
Optimal CD79a staining of the pre-B-ALL using the mAb clone JCB117 within a LD-assay based on HIER in an acidic buffer (BERS1, Leica Biosystems), correctly calibrated titer of the primary Ab (1:50) and Bond Refine (Leica Biosystems) as the detection system. All the neoplastic B-cells display the expected reaction pattern (see Fig. 3a) despite the protocol is based on the less sensitive antigen retrieval solution BERS1. However, optimal results could be obtained on this platform using HIER in BERS1, provided that the titer of the primary Ab was adjusted to the total sensitivity of the protocol employed.

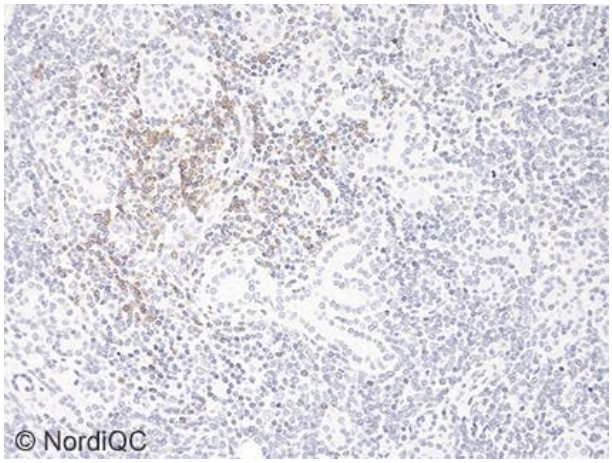


Fig. 6b (x200)
Insufficient CD79 staining of the pre-B-ALL using the same protocol settings as in Fig. 6a except for applying a 4-fold lower concentration of the primary Ab (1:200). All the neoplastic cells are false negative. The normal B-cells intermingling between the neoplastic B-cells and the epithelial cells only display a faint staining reaction. The protocol needs optimization and a simple adjustment of the titer of the primary Ab would probably solve the problem – see Fig. 6a. Importantly, laboratories should always include appropriate controls and stain according to the expected level of antigen densities and hence, not risk misclassification of lymphomas of B-cell type.

MB/LE/SN 10.11.2021