Assessment Run 72 2024
CD8

Purpose
Evaluation of the technical performance, and in particular the level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD8, used for identification of T-cell lymphoproliferative disorders. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD8 (see below).

Material
The slide to be stained for comprised:
1. Appendix, 2. Spleen, 3. B-Chronic Lymphatic Leukemia (B-CLL), 4. Tonsil, 5. T-Cell Lymphoma (TCL), 6. Breast carcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD8 staining as optimal included:

- A strong, predominantly membranous staining reaction of virtually all normal suppressor/cytotoxic T-cells in the appendix, tonsil, spleen and tumour infiltrating cytotoxic T-cells in the breast carcinoma.
- An at least moderate, distinct membranous staining reaction of virtually all the neoplastic T-cells in the TCL.
- An at least moderate, distinct staining reaction of virtually all littoral cells lining sinusoids of the spleen.
- No staining reaction of other tissue/cell structures including normal B-cells (all specimens), epithelial cells of the appendix and the neoplastic cells of the B-CLL (dispersed normal suppressor/cytotoxic T-cells intermingling between the malignant B-cells should be distinctively demonstrated).

KEY POINTS FOR CD8 IMMUNOASSAYS
- The most common mAb clones **C8/144B, 4B11** and the rmAb clones **SP239, SP57** was used by 95% of all participants.
- RTU systems developed for the Autostainer, Omnis, Bond and Benchmark platforms based on the mAb clone **C8/144, 4B11** and the rmAb clone **SP239**, respectively, gave superior results using vendor recommended protocol settings.
- The performance of assays based on the rmAb clones **SP57** and **SP16** was poor, requiring substitution with other more suited antibody clones.
- Tonsil and appendix are not reliable positive tissue controls to monitor the accuracy and precision of CD8 IHC assays – spleen might be a better alternative.

Participation

Number of laboratories registered for CD8, run 72	398
Number of laboratories returning slides	375 (94%)

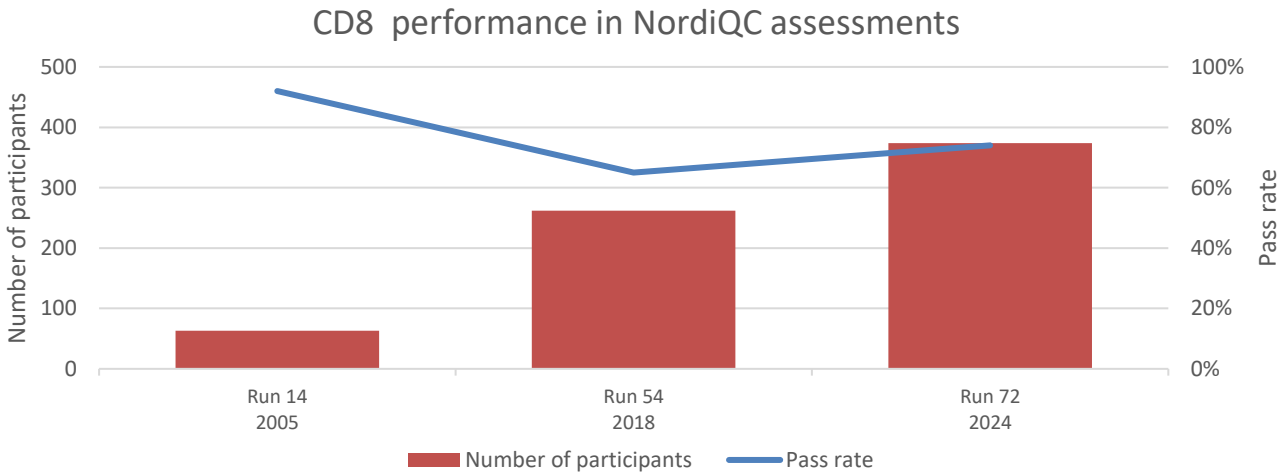
One slide could not be assessed due lack of the appendix (tissue core no 1). All slides returned after the assessment were assessed and participants received advice if the result was insufficient - data from all these outcomes were not included in this report.

Results
374 laboratories participated in this assessment and 275 (74%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Table 1b and 1c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Poor performance of the Ready-To-Use (RTU) product 790-4460, based on rmAb clone **SP57**, on the Benchmark platforms (Ventana/Roche)
- Less successful performance of assays based on the rmAb clone **SP16**
- Use of less sensitive 2-step detection systems e.g. UltraView (Ventana/Roche) and EnVision FLEX (Dako/Agilent)
- Unexplained technical issues

Performance history
This was the third NordiQC assessment of CD8. The pass rate increased compared to the previous run 54, 2018 (see Graph 1).

Graph 1. **Proportion of sufficient results for CD8 in the three NordiQC runs performed**



Controls
Normal tonsil and appendix are recommended as primary positive and negative tissue controls in order to evaluate basic assay performance and analytical sensitivity. Virtually all normal suppressor/cytotoxic T-cells must show a strong, predominantly membranous staining reaction, whereas stromal cells, B-cells and non-cytotoxic T-cells must be negative. In appendix, suppressor/cytotoxic T-cells primarily located in lamina propria mucosa but also situated in the appendiceal epithelium must be strongly stained, whereas the epithelial cells should be negative. No staining reaction should be seen in tonsillar crypt or squamous epithelial cells. However, as tonsil and appendix lack normal tissue structures showing low level of CD8 expression, laboratories should consider including spleen, as littoral cells of the sinusoids should display an at least moderate staining intensity confirming the assays low limit of CD8 demonstration.

Conclusion
The mAb clones **C8/144B**, **4B11**, and the rmAb clone **SP239** are robust antibodies for demonstration of CD8. Irrespective of the clone applied, efficient HIER, use of a sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. Among Ready-to-Use (RTU) systems from the major vendors, and applying vendor recommended protocol settings, the RTU system **PA0183** (Leica Biosystems), **IR623/GA623** (Dako/Agilent) and **790-7176** (Ventana/Roche), based on mAb clones **C8/144B**, **4B11** and the rmAb clone **SP239**, respectively, provided superior performance with an overall pass rate of 99% (89/90) - 89% (80/90) being optimal. For assays based on the rmAB clones **SP57** and **SP16**, the pass rate was only 1% (1/76). These primary antibodies typically gave an aberrant and false positive staining reaction of epithelial cells in e.g. the appendix.

Table 1a. **Overall results for CD8, run 72**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	113	65	35	9	4	88%	58%
Ready-To-Use antibodies	261	142	33	85	1	67%	54%
Total	374	207	68	94	5		
Proportion		55%	18%	25%	1%	74%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CD8, run 72**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **C8/144B**	67 8 1 1 1 1 1 1	Dako/Agilent Cell Marque Epredia Invitrogen Biocare Medical DCS diagnostics BioSB Quartett	47	25	7	2	89%	58%
mAb clone **4B11**	24	Leica Biosystems	16	7	0	1	96%	67%
mAb clone **IHC542**	1	GenomeMe	0	1	0	0	-	-
mAb clone **C8/468**	1	PathSitu Biotechnologies	0	1	0	0	-	-
mAb clone **1A5**	1	BioGenex	0	1	0	0	-	-
rmAb clone **QR068**	2	Quartett	2	0	0	0	-	-
rmAb clone **SP16**	1 1	Cell Marque NeoMarkers	0	0	2	0	-	-
rmAb clone **ZR286**	1	Zeta Corporation	0	0	0	1	-	-
Total	113		65	35	9	4	-	
Proportion			57%	31%	8%	4%	88%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).

Table 1c. **Ready-To-Use antibodies and assessment marks for CD8, run 72**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **4B11 PA0183**[3]	25	Leica Biosystems	22	3	0	0	100%	88%
mAb clone **4B11 PA0183**[4]	13	Leica Biosystems	10	3	0	0	100%	77%
mAb clone **C8/144B IR623**[3]	10	Dako/Agilent	7	3	0	0	100%	70%
mAb clone **C8/144B IR623**[4]	19	Dako/Agilent	9	7	3	0	84%	47%
mAb clone **C8/144B GA623**[3]	36	Dako/Agilent	33	3	0	0	100%	92%
mAb clone **C8/144B GA623**[4]	32	Dako/Agilent	20	5	7	0	78%	63%
mAb clone **C8/144B 108M-97/98**	6	Cell Marque	3	2	1	0	83%	50%
mAb clone **C8/144B 8252-C010**	1	Sakura FineTek	1	0	0	0	-	-
mAb clone **C8/144B BFM-0069**	1	Bioin Biotechnology	1	0	0	0	-	-
mAb clone **C8/144B BSB5169**	1	BioSB	0	1	0	0	-	-
mAb clone **MX117 MAB-1031**	1	Fuzhou Maixin	1	0	0	0	-	-
mAb clone **IHC542 IHC542-7**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **SP239 790-7176**[3]	19	Ventana/Roche	18	0	1	0	95%	95%
rmAb clone **SP239 790-7176**[4]	19	Ventana/Roche	14	5	0	0	100%	74%
rmAb clone **SP57 790-4460**[3]	17	Ventana/Roche	0	0	17	0	0%	0%
rmAb clone **SP57 790-4460**[4]	51	Ventana/Roche	0	0	51	0	0%	0%
rmAb clone **SP16 108R-17/18**	3	Cell Marque	0	0	2	1	-	-
rmAb clone **SP16 MAD-000318QD**	1	Master Diagnostica	0	0	1	0	-	-
rmAb clone **SP16 BRB036**	1	Zytomed Systems	0	0	1	0	-	-
rmAb clone **SP16 GT211202**	1	GeneTech	1	0	0	0	-	-
rmAb clone **C8/1779R API 3219**	1	Biocare Medical	1	0	0	0	-	-
Ab clone **BP6041 I10362E-05**	1	Biolynx Biotechnology	0	0	1	0	-	-
Ab clone **815R4B2 PA577**	1	Abcarta/Abcepta	1	0	0	0	-	-
Total	261		142	33	85	1		
Proportion			54%	13%	33%	0%	67%	

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)

Detailed analysis of CD8, Run 72
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **C8/144B**: Protocols with optimal results were all based on Heat Induced Epitope Retrievel (HIER) in an alkaline buffer using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (10/18)*, Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (2/5), Cell Conditioning 1 (CC1; Ventana/Roche) (33/53), HIER buffer H (Epredia) (1/1) and Tris-EDTA/EGTA buffer pH 9 (1/2) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 61 of 68 (90%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **4B11**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) (2/2), BERS2 (Leica Biosystems) (5/9), PRIME Epitope Retrieval Solution 2 (PERS2;Leica Biosystems) (1/3), Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (1/1) and CC1 (Ventana/Roche) (7/9) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 19 of 19 (100%) laboratories produced a sufficient staining result.

rmAb clone **QR068**: The two protocols with an optimal result were based on HIER using either BERS2 (Leica Biosystems) or CC1 (Ventana/Roche) as retrieval buffer. The rmAb was diluted 1:100 and BOND Refine (Leica Biosystems, DS9800) or OptiView (Ventana/Roche, 760-700) were used as detection system.

Table 2. **Proportion of optimal results for CD8 for the most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH6.1	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 **C8/144B**	1/4**	-	9/12 (75%)	-	30/47 (64%)	-	2/5 (40%)	0/2
mAb clone **4B11**	-	0/1	1/1	-	7/8 (88%)	-	4/9 (44%)	1/1

* 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)
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra
3) Bond III/PRIME

Ready-To-Use antibodies and corresponding systems (≥5 protocols).
mAb clone **4B11**, product no. **PA0183**, Leica Biosystems, Bond III/PRIME:
Protocols with optimal results were typically based on HIER using BERS2 or PERS2 (efficient heating time 20 min. at 100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 32 of 32 (100%) laboratories produced a sufficient staining result – 91% (29/32) being optimal.

mAb clone **C8/144B**, product no. **IR623**, Dako/Agilent, Autostainer:
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 95-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000) or EnVision FLEX+ (K8000+K8021) as detection systems. Using these protocol settings, 16 of 16 (100%) laboratories produced a sufficient staining result – 63% (10/16) being optimal.

mAb clone **C8/144B**, product no. **GA623**, 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), 10 min. incubation of the primary Ab and EnVision FLEX+ (GV800/823+GV821) as detection systems. Using these protocol settings, 36 of 36 (100%) laboratories produced a sufficient staining result – 92% (33/36) being optimal.

rmAb clone **SP239**, product no. **790-7176**, Ventana/Roche, BenchMark Ultra/Ultra PLUS/GX:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 12-32 min. incubation of the primary Ab and OptiView (760-700) or UltraView (760-500) as detection system. Using these protocol settings, 35 of 36 (97%) laboratories produced a sufficient staining result – 83% (30/36) being optimal.

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 CD8 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb C8/144B **IR623**	100% (10/10)	70% (7/10)	78% (7/9)	44% (4/9)
Dako Omnis mAb C8/144B **GA623**	100% (36/36)	92% (33/36)	73% (19/26)	54% (14/26)
Leica BOND III/PRIME mAb 4B11 **PA0183**	100% (25/25)	88% (22/25)	100% (12/12)	75% (9/12)
VMS Ultra/PLUS/GX rmAb SP239 **790-7176**	95% (18/19)	95% (18/19)	100% (19/19)	74% (14/19)
VMS Ultra/PLUS/GX rmAb SP57 **790-4460**	0% (0/17)	0% (0/17)	0% (0/51)	0% (0/51)

* 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 are integrated.

Comments
In this third assessment of CD8, the prevalent feature of an insufficient result was characterized by a false positive staining result of appendiceal epithelium, primarily related to the use of the rmAb clones SP57 and SP16 accounting for 74% (73/99) of all insufficient results. One protocol based on the rmAb clone ZR286 also provided a false positive staining reaction. In the remaining 25% (25/99) of insufficient results, false negative or too weak staining reactions were seen often in combination with uneven and/or excessive counter/background staining - compromising interpretation of the specific signal for CD8.
Most laboratories were able to demonstrate CD8 in high-level antigen expressing cells, such as normal cytotoxic T-cells in all specimens, whereas demonstration of CD8 in the neoplastic T-cells of the TCL and the littoral cells lining sinusoids of the spleen was more challenging, requiring an optimally calibrated protocol.

Used as concentrates within laboratory developed assays (LD), the mAb clones **C8/144B and 4B11** were the most widely used antibodies for demonstration of CD8, both providing a relatively high pass rate of 89% (72/81) and 96% (23/24), respectively (see Table 1b). Performance characteristic on the respective automatic platforms from the major vendors are outlined in Table 2, and as shown for the mAb clone **C8/144B**, optimal results could be obtained on all main fully automated platforms. The clone was most successful on Omnis providing the highest proportion of optimal results when applied in the "recommended/optimal" dilution range 1:25-200. Within LD-assays, nine protocols based on the mAb clone **C8/144B**, were assessed as insufficient and the main parameters causing inferior performance was related to the use of less efficient HIER in acidic buffer (one protocol), too diluted primary antibody (one protocol) and four protocols were based on the less sensitive 2-step detection systems (UltraView and EnVision FLEX). In the remaining three insufficient protocols, no parameters could be identified explaining the less successful performance and thus, categorized as "unexplained technical issues".

As seen in the previous run 54 for CD8, the mAb clone **4B11** used as concentrate within LD-assay, gave a high proportion of sufficient and optimal results, 96% (23/24) and 67% (16/24), respectively. Optimal results could be obtained on all fully automated platforms with highest success rate on the Benchmark platforms (see Table 2). All optimal results (16/16) were based on protocols using a 3-step detection system as Bond Refine, OptiView or Envision FLEX+, the average dilution was 1:92 (range 1:25-200) and HIER in an alkaline buffer were used by 94% (15/16) of the laboratories. Seven protocols gave a result scored as "good", based on identical protocol settings as described above, but downgraded due to excessive background staining, primarily related to use of mAb clone **4B11** on the BOND-III and BOND-PRIME platforms. This problem is often seen in relation to use of the covertile-technology on these platforms. The reason for discrepancies in performance between laboratories of the BOND platforms is difficult to elucidate upon, but could reflect different laboratory practices e.g., maintenance of the

covertiles or how often these are substituted with new ones. The one protocol assessed as insufficient used a 2-step detection system (EnVision FLEX).

70% (261/374) of the laboratories used an RTU format for the demonstration of CD8. This is an increase compared to the former run 54, 2018 in which 58% (151/262) of the participants applied a RTU format. In this assessment, and using vendor recommended protocol settings (VRPS), the RTU systems **PA0183** (Leica Biosystem/Bond platforms), **IR623/GA623** (Dako/Agilent Autostainer and Omnis) and **790-7176** (Ventana/BenchMark platforms) based on the mAb clones **C8/144B**, **4B11** and the rmAb clone **SP239**, respectively, provided superior results (see Table 3). Grouped together, the pass rate was 99% (89/90) of which 89% (80/90) were assessed as optimal. These RTU systems were very robust giving reproducible high quality staining results and was proven to be applied as "true plug-and-play systems" for routine purpose.
Applying laboratory modified protocol settings (LMPS) did not improve the staining quality for these RTU systems, and in fact, a decrease in optimal performance was observed for all these RTU systems using modifications to the protocol recommendations given by the respective vendors (see Table 3). In line with this observation, it was shown that protocols for the Dako/Agilent RTU system **GA623** developed for the Omnis being modified by exchanging the recommended EnVision FLEX+ (with mouse linker) to Envision FLEX provided an inferior performance as only 50% (6/12) of these protocols gave a sufficient result – only 17% (2/12) being optimal. In contrast, applying EnVision FLEX+ as the detection system, the proportion of sufficient and optimal results increased significantly to 98% (49/50) and 90% (45/50), respectively. This single and simple change of protocol settings (omitting the mouse linker) had a dramatically negative effect on the results and indicates, that participants should follow recommendations given by Dako/Agilent for the CD8 GA623 assay.A significant proportion of laboratories used the RTU formats **IR623** and **GA623** off-label on non-validated platforms giving at pass rate 94% (15/16). This procedure is in general not recommendable, unless each laboratory has performed an extensive and thorough validation process, documenting the accuracy and analytical sensitivity/specificity of the test.

The Benchmark users can select from two RTU products from the same vendor Ventana/Roche, **790-7176** or **790-4460**, based on the rmAb clones **SP239** and **SP57**, respectively. In total, 106 protocols were based on these two RTU systems of which the minority of laboratories used the **790-7176** (see Table 1c or Table 3). This recently launched RTU system **790-7176**, seems promising and was found very robust for demonstration of CD8, providing a high proportion of sufficient and optimal result applying both UltraView or Optiview as the detection system. However, the proportion of optimal results was slightly lower if UltraView was used as detection system, 75% (15/20), compared to Optiview giving 94% (15/16) optimal results. The one protocol assessed as insufficient (see Tabel 1c) used exactly same protocol settings as protocols giving an optimal result, and thus, categorized as "unexplained technical issues".

In comparison to all other RTU systems from the major vendors, and despite using a variety of protocol settings (both VRPS or LMPS), the RTU system **790-4460** (Ventana/Roche), based on the rmAb clone **SP57** developed for the Benchmark platforms, provided inferior results with a pass rate of 0% (0/68).

The unsatisfactory and high proportion of insufficient results were caused by a false positive staining reaction of epithelial cells e.g. appendiceal epithelium but also observed in crypt epithelium of the tonsillar tissue.

This aberrant staining result has also been described in the previous report run 54, and in total 141 protocols based on the rmAb clone **SP57** have been assessed by NordiQC, of which none were sufficient. Thus, laboratories are encouraged to substitute this unsuccessful RTU system with the RTU system **790-7176** based on the rmAb clone **SP239**, giving excellent results. Also, the vendor should consider discontinuing the RTU product **790-4460**, and thereby helping customers avoiding making wrong decisions and choosing the superior RTU system **790-7176**.

As for the rmAb clone **SP57**, the same problem was observed with rmAb clone **SP16** (both as concentrate and RTU formats) giving false positive results, and only one of eight protocols in this assessment was giving a sufficient score (optimal). At present, it is still enigmatic why one laboratory received an optimal score and therefore difficult to unravel. The same aberrant problematic staining patterns were also observed in run 54 (2018), and laboratories/vendors are as for rmAb clone **SP57**, encouraged to substituted with an antibody, providing the correct level of analytical sensitivity and specificity for demonstration of CD8.

This was the third assessment of CD8 in NordiQC (see Graph 1). The pass rate increased slightly to 74% in this assessment compared to 65% in the previous run 54 (2018). In this assessment, the most common

cause for an insufficient staining result was related to the use of the rmAb clones **SP57** or **SP16**, accounting for 76% (75/99) of all insufficient results - typically giving false positive staining reaction of columnar epithelial cells in the appendix. Omitting protocols based on the rmAb clones **SP57** and **SP16** from the data set in this assessment, the overall pass rate would have been 92% (274/298) – 69% (206/298) being optimal. These data support, that selection of a good antibody clone is mandatory for optimal performance of the assay. Importantly, and for in-house choice of an antibody clone, protocol settings must be carefully calibrated according to the expected reaction patterns of the recommended positive and negative control materials.

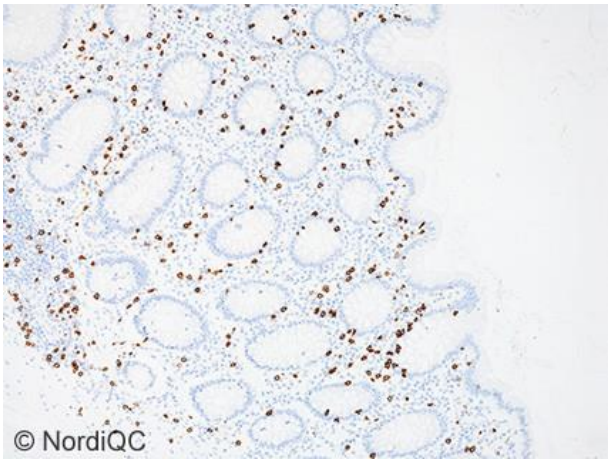


Fig. 1a (x100)
Optimal CD8 staining reaction of the appendix using the RTU system (Dako/Agilent, **GA623**) based on mAb clone **C8/144B** on the Omnis, following recommendations provided by the vendor: HIER (30 min. at 97°C) in TRS pH High, incubation time in primary Ab for 10 min and Envision Flex+ as detection system – Same protocol used in Figs. 2a – 6a.
Virtually all cytotoxic T-cells situated in lamina propria mucosa show a strong, distinct membranous staining reaction. Dispersed cytotoxic T-cells are also demonstrated in the appendiceal epithelium. Epithelial cells are negative as expected.

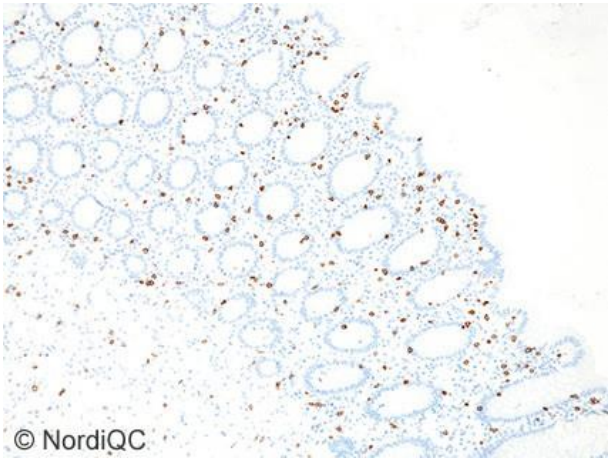


Fig. 1b (x100)
CD8 staining of the appendix using the same RTU system as in Fig. 1a, but without the mouse linker (Envision Flex instead of EnVision Flex+) – same protocol used in Figs. 2b – 6b.
The protocol gave the expected reaction pattern although with weaker intensity. However, demonstration of littoral cells of the spleen, the neoplastic T-cells of the TCL and tumor infiltrating cytotoxic T-cells in breast carcinoma (or the B-CLL) were more challenging (see Figs. 2a – 6b).

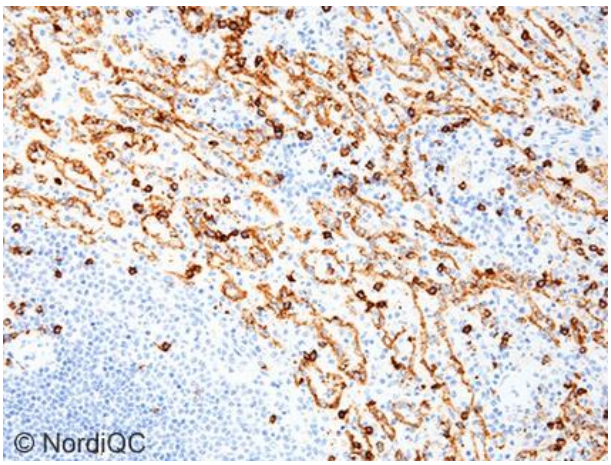


Fig. 2a (x200)
Optimal CD8 staining reaction of the spleen using same protocol as in Fig. 1a. All littoral cells lining the sinusoids display a moderate and distinct staining reaction, whereas T-cells with cytotoxic activity are strongly demonstrated.

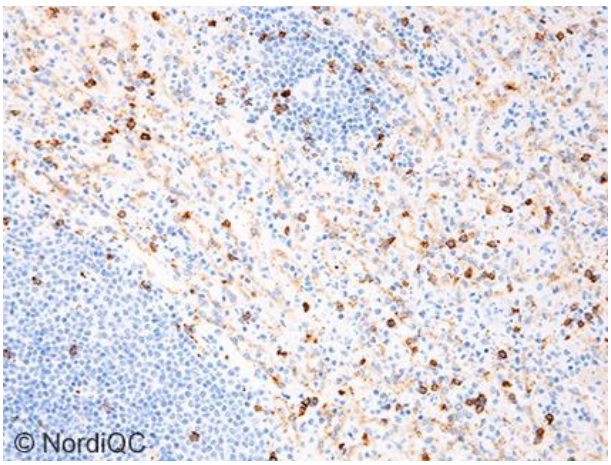


Fig. 2b (x200)
CD8 staining reaction of the spleen using the same protocol as in Fig. 1b. The staining intensity of the littoral cells is too weak compared to the result seen in Fig. 2a. As "appendix and tonsil control material" do not display low level of expression for CD8, laboratories should consider, especially in the validation process but also as daily control for CD8, to include normal spleen as control tissue to monitor precision of the IHC assay with focus on low limit of demonstration.

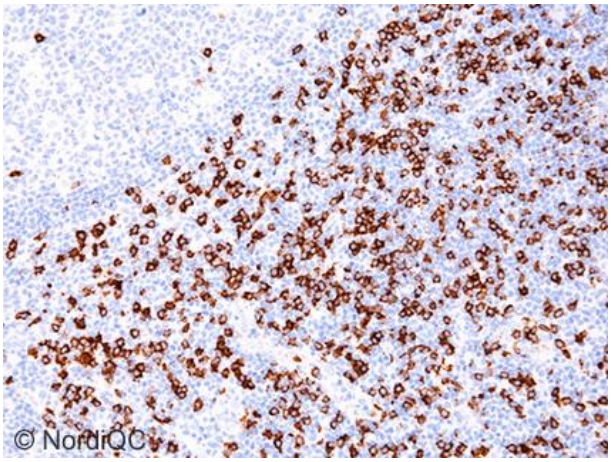


Fig. 3a (x200)
Optimal CD8 staining reaction of the tonsil using same protocol as in Figs. 1a and 2a. All the cytotoxic T-cells display a strong distinct membranous staining reaction, whereas non-cytotoxic T-cell and B-cells are negative as expected.

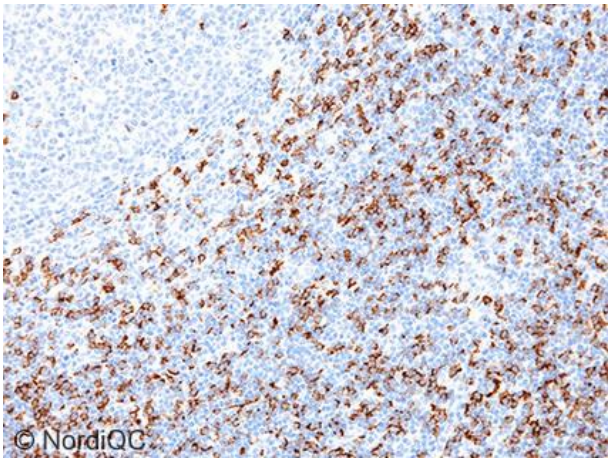


Fig. 3b (x200)
CD8 staining reaction of the tonsil using same protocol as in Figs. 1b and 2b. The staining intensity is too weak, and the positive cytotoxic T-cells display a "irregular" and inconsistent membranous reaction pattern – compare with the protocol in Fig 3.a.

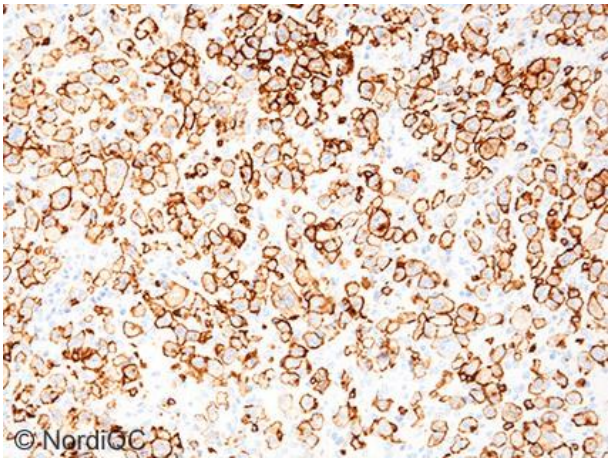


Fig. 4a (x100)
Optimal CD8 staining reaction of the TCL using same protocol as in Figs. 1a – 3a. Virtually all neoplastic T-cells are strongly positive.

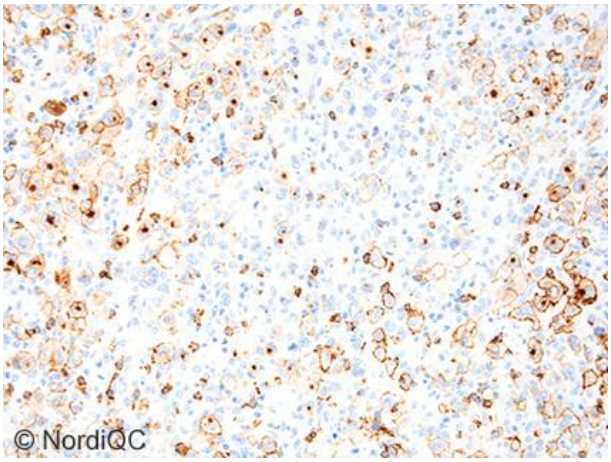


Fig. 4b (x100)
CD8 staining reaction of the TCL using same protocol as in Figs. 1b – 3b. The staining intensity and proportion of positive neoplastic T-cells are significantly reduced - compare with Fig.4a.

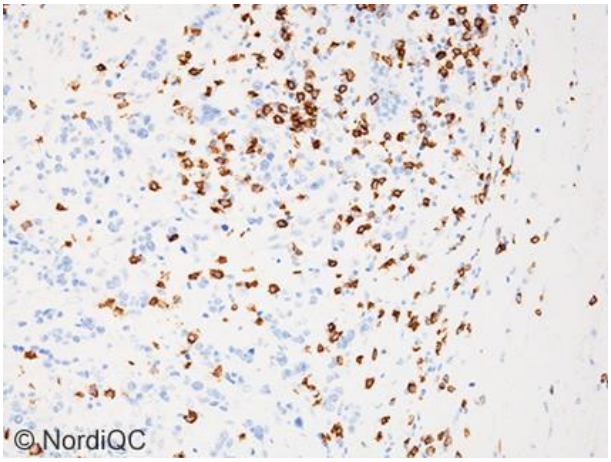


Fig. 5a (x200)
Optimal CD8 staining reaction of the breast carcinoma using the same protocol as in Figs. 1a – 4a. Virtually all tumor infiltrating cytotoxic T-cells display a strong, distinct membranous staining reaction, whereas the neoplastic cells of the breast carcinoma as expected are negative.

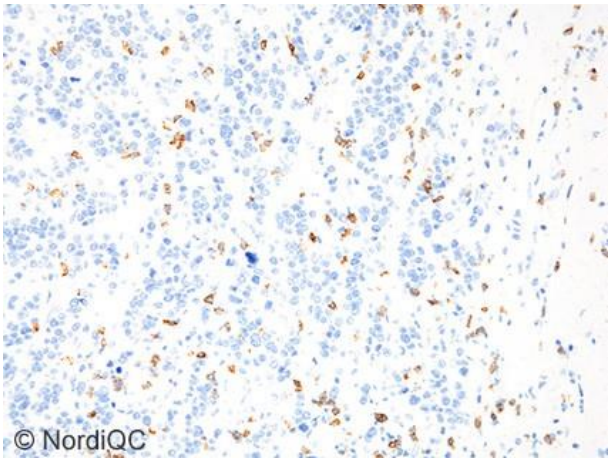


Fig. 5b (x200)
Insufficient CD8 staining reaction of the breast carcinoma using same protocol as in Figs. 1b – 4b. The majority of the tumor infiltrating cytotoxic T-cells are false negative or too weakly demonstrated - compare with the optimal result in Fig. 5a.

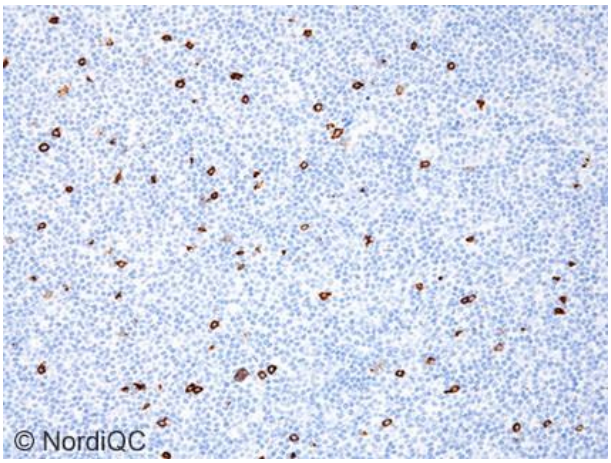


Fig. 6a (x200)
Optimal CD8 staining reaction of the B-CLL using the same protocol as in Figs. 1a – 5a. All normal cytotoxic T-cells intermingling between the neoplastic B-cells are strongly positive.

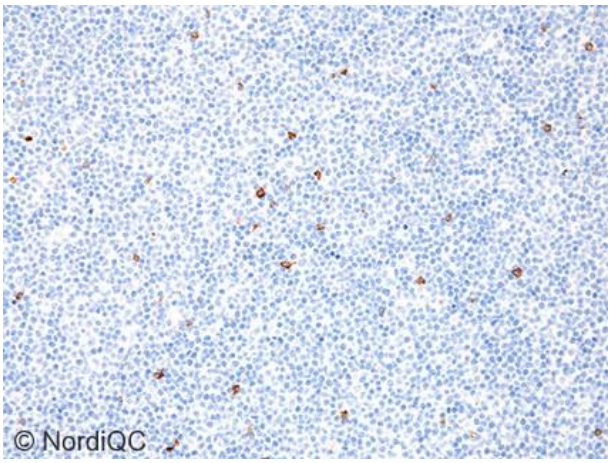


Fig. 6b (x200)
CD8 staining reaction of the B-CLL using the same insufficient protocol as in Figs. 1b – 5b. Staining intensity and proportion of positive normal cytotoxic T-cells are significantly reduced – compare with the optimal result in Fig. 6a.

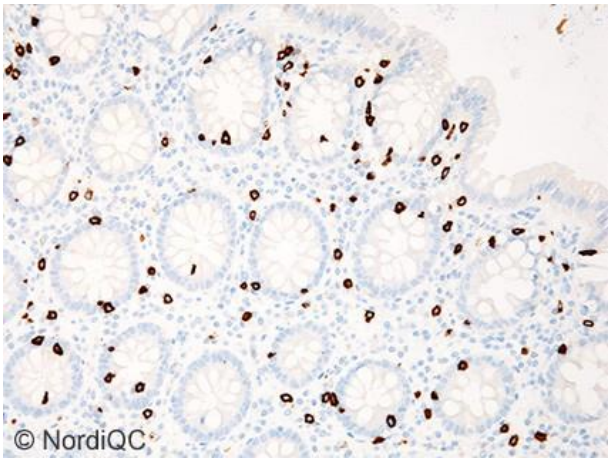


Fig. 7a (x200)
Optimal CD8 staining reaction of the appendix using the RTU system **790-7176** (Ventana/Roche) based on the rmAb clone **SP239** on the BenchMark Ultra platform (Ventana), HIER in CC1 for 64 min., incubation time in primary Ab for 12 min. and OptiView as detection system.
This protocol gave the expected reaction pattern in all cores - see examples in Figs. 1a – 6a.

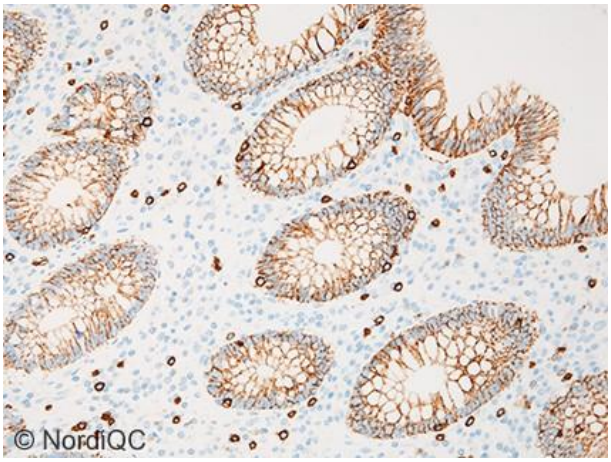


Fig.7b (x200)
CD8 staining of the appendix using the RTU product **790-4460** based on the rmAb clone **SP57** on the Benchmark platforms (Ventana/Roche). All assays based on this clone, gave an aberrant and a false positive staining reaction of epithelial cells in the appendix. Due to this problem, participants should substitute this inferior format with the high quality RTU system described in Fig. 7a.
As for the assays based on rmAb clone SP57, the rmAb clone SP16 provided similar aberrant false positive reactions, and thus, laboratories using this antibody clone are also encouraged to change to a robust and specific antibody.

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