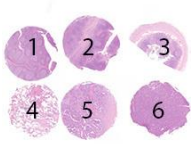
Assessment Run 61 2021
CD56

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD56, typically identifying neuroendocrine tumours/carcinomas or subtypes of T-cell lymphomas in the characterization of tumours of unknown origin. CD56 is also useful in discrimination of reactive from neoplastic plasma cell proliferations. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for CD56 (see below).

Material
The slide to be stained for CD56 comprised:



1-2. Tonsil, 3. Appendix, 4. Neuroendocrine tumour, 5. Colon Adenocarcinoma, 6. Plasmacytoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD56 staining as optimal included:

- A strong, predominantly membranous staining reaction of peripheral nerves (all specimens).
- A strong, distinct membranous staining reaction of the interfollicular NK-cells and a subset of T cells (CD4/CD8 double positive) in the tonsils.
- A weak to moderate, predominantly membranous staining reaction of the majority of neoplastic cells in the neuroendocrine tumour.
- A strong, distinct membranous staining reaction of virtually all neoplastic cells in the plasmacytoma.
- An at least weak staining reaction in dispersed fibroblastic reticular cells in tonsils, appendix and colon adenocarcinoma.
- No staining reaction of normal epithelial cells in appendix and tonsil and of the neoplastic cells in the colon adenocarcinoma.

A weak to moderate staining reaction of smooth muscle cells surrounding vascular structures in the tonsils and appendix, was accepted. This staining pattern was only seen with the rabbit monoclonal antibody (rmAb) clone MRQ-42. Currently, the NordiQC organization is unable to explain for this deviating reaction pattern due to limited literature on this field. However, CD56 has been reported positive in smooth muscle cell tumours, and thus, it cannot be excluded that these tissue structures could be positive applying high affinity antibodies against CD56.

Participation

Number of laboratories registered for CD56, run 61	372
Number of laboratories returning slides	324 (87%)

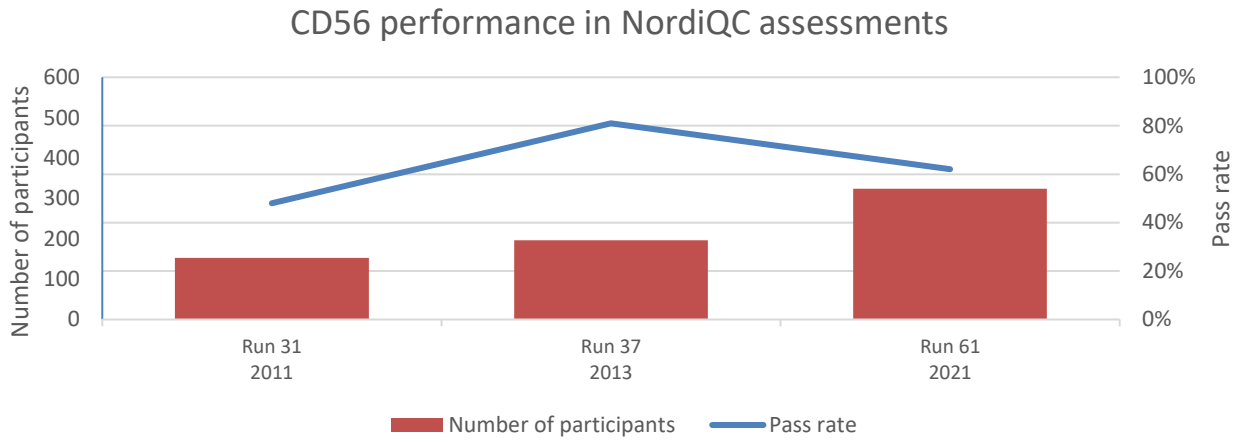
Results
At date of assessment, only 87% of the participants had returned the circulated NordiQC slides, this was in particular subscribed to the Covid-19 pandemic and associated postal delays.
All slides returned after the assessment were assessed, and laboratories received advice if assessment score was insufficient, but primary data was not included in this report.

324 laboratories participated in this assessment and 62% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Less successful performance of the mouse monoclonal Ab (mAb) clone 123C3, 123C3.D5 and CD564
- Inappropriate use of the Ready-to-use (RTU) format IR/IS628 (Dako) on non-validated platforms e.g., Omnis
- Too low concentration of the primary antibody or too short incubation time
- Less sensitive detection systems
- Inefficient Heat Induced Epitope Retrieval (HIER) – too short time or use or acidic buffer.

Performance history
This was the third NordiQC assessment of CD56. A significant decrease in pass rate was observed compared to previous run 37 (2013) (see Figure 1). The causes for the declined pass rate could be correlated to several parameters impacting the overall performance of the assays (see comments).

Figure 1. **Proportion of sufficient results for CD56 in the three NordiQC runs performed**



Conclusion
The mAb clones 123C3, 1B6, CD564, IHC056 and MX039, and the rmAb clone MRQ-42 could all be used to obtain an optimal staining for CD56. In this assessment, the rmAb clone MRQ-42 provided superior performance, both as concentrate and RTU formats, and 99% (136/137) of the protocols were assessed as sufficient. This clone was very robust and provided a high proportion of optimal results on all main IHC platforms and by several different protocol settings. In contrast the mAb clones 123C3 and CD564 were less successful and in total only 33% of the protocols (54/164) provided a sufficient result and only 5% (8/164) being optimal. Especially mAb clone 123C3 was found inferior when applied on the fully automated platforms as BenchMark (Ventana/Roche) and Omnis (Dako/Agilent). This was in particular observed for laboratories using the RTU format IR/IS628 of mAb clone 123C3, tailored for the Autostainer, on the Omnis platform as no protocols (37/37) gave a sufficient result.
Tonsil is recommended as positive and negative tissue control: Virtually all the interfollicular NK-cells and CD4/CD8 double positive T-cells must show a strong, predominantly membranous staining reaction, while all other subtypes of lymphocytes should be negative.

Table 1. **Antibodies and assessment marks for CD56, Run 61**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **123C3**	25 1	Dako/Agilent Genemed	4	8	8	6	46%	15%
mAb clone **123C3.D5**	3 1 1 1	Cell Marque Diagnostic Biosystems BioSB Zeta Corporation	0	1	1	4	17%	0%
mAb clone **CD564**	18 1	Leica Biosystems Monosan	2	5	9	3	36%	11%
mAb clone **1B6**	3	Leica Biosystems	2	1	0	0	-	-
mAb clone **BC56C04**	3	Biocare Medical	0	1	1	1	-	-
mAb clone **56C04**	1	Thermo F. Scientific	0	0	0	1	-	-
mAb clone **123A8**	1	BioGenex	0	0	0	1	-	-
rmAb clone **MRQ-42**	49	Cell Marque	41	7	1	0	98%	84%
rmAb clone **RCD56**	1	Zytomed Systems	0	1	0	0	-	-
Ready-To-Use antibodies								
mAb clone **123C3, IR628/IS628**[3]	15	Dako/Agilent	0	7	6	2	47%	0%
mAb clone **123C3, IR628/IS628**[4]	55	Dako/Agilent	0	8	27	20	44%	0%
mAb clone **123C3,**	2	Ventana/Roche	0	0	2	0	-	-

790-4465[3]								
mAb clone **123C3, 790-4465[4]**	25	Ventana/Roche	0	5	9	11	20%	0%
mAb clone **123C3, MAD-000749QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **123C3.D5, 156M-87/88**	2	Cell Marque	0	0	1	1	-	-
mAb clone **BC56C04, PM164**	1	Biocare Medical	0	0	1	0	-	-
mAb clone **C5A2, CCM-0662**	1	Celnovte Biotechnology	0	0	1	0	-	-
mAb clone **CD564, PA0191[3]**	6	Leica Biosystems	0	4	2	0	67%	0%
mAb clone **CD564, PA0191[4]**	14	Leica Biosystems	1	8	3	2	64%	7%
rmAb clone **IHC066, IHC066**	1	GenomeMe	1	0	0	0	-	-
mAb clone **MX039, MAB-0743**	2	Fuzhou Maixin Biotech	2	0	0	0	-	-
rmAb clone **MRQ-42, 760-4596[3]**	0	Ventana/Roche	0	0	0	0	-	-
rmAb clone **MRQ-42, 760-4596[4]**	68	Ventana/Roche	49	19	0	0	100%	72%
rmAb clone **MRQ-42, 156R-97/98**	16	Cell Marque	11	5	0	0	100%	69%
rmAb clone **MRQ-42, 8274-C010**	4	Sakura Finetek	2	2	0	0	-	-
rmAb clone **RCD56, RBG050**	1	Zytomed Systems	1	0	0	0	-	-
Total	324		117	83	72	52	-	
Proportion			36%	26%	22%	16%	62%	

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 CD56, Run 61
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **123C3**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS, Dako/Agilent) pH 9 (3-in-1) (3/13)* or Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 5 of 7 (71%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **CD564**: Protocols with optimal results were based on HIER in TRS High pH (3-in-1) (Dako/Agilent) (1/1) or Cell Conditioning 1 (CC1, Ventana/Roche) (1/9) as retrieval buffer. The mAb was diluted in the range 1:20-1:100. Using these protocol settings, 4 of 10 (40%) laboratories produced a sufficient staining result.

mAb clone **1B6**: Protocols with optimal results were based on HIER in CC1 (Ventana/Roche) (1/1) or Bond Epitope Retrieval Solution 2 (BERS2, Leica) (1/1) as retrieval buffer. The mAb was diluted in the range 1:100-1:200. Using these protocol settings, 3 of 3 (100%) laboratories produced a sufficient staining result.

rmAb clone **MRQ-42**: Protocols with optimal results were based on HIER in CC1 (Ventana/Roche) (29/35), TRS High pH (3-in-1) (Dako/Agilent) (7/8), BERS2 (Leica) (3/3), TRS Low pH (3-in-1) (Dako/Agilent) (1/1) or BERS1 (Leica) (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:500. Using

these protocol settings, 40 of 40 (100%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for CD56 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica 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	ER2 pH 9.0	ER1 pH 6.0
mAb clone **123C5**	0/2**	0/1	2/4	-	0/5 (0%)	-	-	1/1
rmAb clone **MRQ-42**	1/1	-	5/5 (100%)	1/1	24/28 (86%)	-	2/3	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).

Ready-To-Use antibodies and corresponding systems
rmAb clone **MRQ-42**, product no. **760-4596**, 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), 16 min. incubation of the primary Ab and OptiView (760-700) or UltraView with or without amplification (760-099/860-099 or 760-080, respectively) as detection system. Using these protocol settings, 28 of 28 (100%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **MRQ-42**, product no. **8274-C010**, Sakura FineTek, Tissue-Tek Genie Advanced:
Protocols with optimal results were based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (efficient heating time 45 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit (8826-K250) as detection system. Using these protocol settings, 4 of 4 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **CD564,** product.no. **PA091**, Leica Biosystems, BOND III/MAX:
One protocol with an optimal result was based on HIER in BERS2 (efficient heating time 20 min. at 99-100°C), 15 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 5 of 7 (71%) laboratories produced a sufficient staining result.

mAb clone **MX039**, product no. **MAB-0743**, Fuzhou Maixin Biotech, Titan S:
Protocols with optimal results were based on HIER using High pH buffer (Fuzhou Maixin, DNS-0811) (efficient heating time 20 min. at 99°C), 30 min. incubation of the primary Ab and Titan Super Detection Kit (TT-0805) as detection system. Using these protocol settings, 2 of 2 (100%) laboratories produced a sufficient staining result (optimal or good).

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 CD56 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb 123C3 **IR/IS628**	47% (7/15)	0% (0/15)	44% (8/18)	0% (0/18)
VMS Ultra/XT/GX mAb 123C3 **790-4465**	0/2	0/2	20% (5/25)	0% (0/25)
Leica Bond III/MAX mAb CD564 **PA0191**	67% (4/6)	0% (0/6)	62% (8/13)	8% (1/13)
VMS Ultra/XT/GX rmAb MRQ-42 **760-4596**	0/0	0/0	100% (68/68)	72% (49/68)

* 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 concordance with the previous NordiQC assessments for CD56, the prevalent feature of an insufficient staining was a general too weak or false negative staining reaction of cells and structures expected to be demonstrated. This was observed in 98% (121/124) of the insufficient results. Almost all laboratories could detect CD56 in peripheral nerve cells of the appendix, whereas demonstration of CD56 in NK-cells and CD4/CD8 double positive T-cells of the tonsils, the fibroblastic reticular cells in e.g., tonsils and in the neoplastic cells of the neuroendocrine tumour or plasmacytoma was more challenging, requiring an optimally calibrated protocol.

The mAb clones 123C3, CD546 and the rmAb clone MRQ-42 were the most widely used antibodies for demonstration of CD56 and applied by 93% (301/324) of the laboratories (see Table 1). Used as concentrated format within laboratory developed (LD) assays, the mAb clone 123C3 gave a pass rate of 46% (12/26) of which only 15% (4/26) of the protocols gave an optimal result. The most prevalent feature of an insufficient result, and as described in the previous report (Run 37), was related to inferior performance of the primary Ab on the Benchmark platforms (Ventana/Roche) as only 11% (1/9) of protocols based on clone 123C3 provided a sufficient result (good). The remaining assays providing insufficient results, were characterized by usage of low sensitive protocol settings as too diluted primary Ab often used in combination with a 2-step polymer detection system e.g., Envision Flex (Dako/Agilent). Although the proportion of sufficient results were relative high (75%, 6/8) for protocols applied on the fully automated platform Omnis (Dako/Agilent), only 25% (2/8) provided an optimal result, indicating that the mAb clone 123C3 could be challenging on this platform as well. However, for optimal performance the protocols must be careful calibrated using highly sensitive protocol settings as efficient HIER in an alkaline buffer, high concentration of the primary Ab (e.g., dilution range of 1:25-50) and preferably a 3-step polymer detection system.

For laboratories using the mAb clone 123C3.D5, both as concentrate and RTU format, the same performance/pattern was seen as for the mAb clone 123C3 and none (5/8) of the protocols applied on Benchmark were able to produce a sufficient result.

Used within a LD assay, the mAb clone CD564 gave a pass rate of 36% (7/19) of which only 11% (2/19) were assessed as optimal. Optimal results were obtained using efficient HIER in an alkaline buffer, a high concentration of the primary Ab (dilution range 1:20-100), prolonged incubation time in primary Ab (up to 120 min.) and a sensitive 3-step polymer/multimer detection system (EnVision Flex+ (Dako/Agilent) or OptiView (Ventana/Roche)). The primary causes of insufficient results were use of protocols with low analytical sensitivity, as inefficient HIER (acidic buffer or too short HIER time), too diluted primary ab, too short incubation time in primary Ab or use of a 2-step multimer/biotin-based detection system - often applied in combination. In the previous assessment (Run 37), the mAb clone CD564 provided excellent results, both within LD assays and the RTU system PA0191 (Leica), which is in disagreement with the results obtained in this assessment, only providing 51% (20/39) sufficient results for all protocols based on this clone. The reasons for this divergent result are difficult to elucidate upon and currently not known, but a significant increase in new laboratories attending the external quality program for the first time and using clone CD564 being increased from 15 to 39 participants in Run 37 and Run 61, respectively, together with challenging tissue circulated (e.g., the neuroendocrine tumuor), could be a part of the explanation.
As shown in Table 1, the use of rmAb clone MRQ-42 within a LD assay provided superior results giving a pass rate of 98% (48/49) of which 84% (41/49) were optimal. Also, the rmAb clone MRQ-42 was the only antibody that could provide optimal results on the four main automated platforms (see Table 2). The primary Ab is very robust, and several protocol settings could be used to obtain an optimal result. However, the vast majority of laboratories applied efficient HIER in an alkaline buffer (34/36) and used a 3-step polymer/multimer as detection system (27/36) e.g., Envision Flex+ (Dako/agilent) or OptiView (Ventana/Roche). Using these settings as protocol backbone the primary Ab concentration could be used in the range of 1:25 – 1:1000 to generate an optimal result. The one laboratory obtaining an insufficient result (borderline), used the primary Ab too diluted (1:2000) in combination with the less sensitive detection system Ultraview (Ventana/Roche).

In total, 66% (215/324) of the laboratories used a RTU format. Following vendor recommended protocol settings (VRPS), the Dako/Agilent RTU system (IR/IS628) based on the mAb clone 123C3, tailored for the Dako Autostainer, gave a low pass rate of 47% (7/15) and none of the protocols gave an optimal result (see Table 3), despite being based on protocol settings providing a high level of sensitivity such as efficient HIER in TRS pH 9 and the 3-step polymer detection system Envision Flex+. No single parameter could be identified unravelling the cause for the less successful performance of the assay. As mentioned above, especially the neuroendocrine tumour was challenging and optimal staining of the neoplastic cells was only seen, if all technical protocol parameters were carefully adjusted.

For laboratories using the same RTU product, and applying Laboratory Modified Protocol Settings (LMPS), the pass rate was 44% (8/18) – none being optimal. A significant proportion of participants (n=37) used the RTU format IR/IS628 on the Dako Omnis, most likely due to lack of a RTU alternative to this instrument, and none were able to produce a sufficient result (optimal or good). This observation, and in accordance with the results obtained with the concentrated formats, indicate that the use of the mAb clone 123C3 might be difficult to optimize on the Omnis. In this context it has to be emphasized that, a "direct" transfer of a RTU product developed for the Autostainer to the Omnis platform should be avoided and require a careful validation by the laboratory. Adjustments of key parameters as HIER, incubation time of the primary antibody and choice of detection system is typically needed to provide the correct analytical accuracy for the IHC test, and can at the end, be impossible to achieve working with low affinity antibodies on certain platforms (e.g., Omnis or Benchmark).

The RTU format 760-4465 (Ventana/Roche) also based on the mAb clone 123C3, provided a low proportion of sufficient results (19%, 5/27) of which none were optimal, and only two participants applied VRPS (see Table 3). Overall, as described above and observed in previous runs for CD56 the performance of this RTU system is challenged by the inferior performance of the Ab on fully automated IHC platforms and laboratories are advised to substitute this product with the RTU format 760-4596 based on the rmAb clone MRQ-42, providing significantly better results (see below/Table 3).

Compared to the RTU systems above, the Leica RTU format PA0191 based of mAb clone CD564 provided a higher, but still low, pass rate of 63% (12/19) of which only 5% (1/19) were optimal. There was no significant difference applying VRPS or LMPS (see Table 3) and e.g. using a more efficient HIER in BERS2, instead of HIER in BERS1 as recommended by Leica, only slightly improved the overall performance from 66% (4/6) to 71% (5/7) sufficient results, despite applying identical protocol settings. Compared to the former Run 37, no parameters could be identified, explaining for the decrease in performance seen in this assessment other than the causes discussed above for the concentrated format.

In this assessment, the RTU system 760-4596 (Ventana/Roche) based on the rmAb MRQ-42 provided superior performance compared to all other RTU systems from the major vendors. The pass rate was 100% (68/68) and 72% (49/68) were assessed as optimal. However, none of the protocols were based on VRPS (see Table 3). The RTU systems is very robust, and several protocol settings could be used to obtain an optimal result (see Ready-to-Use antibodies and corresponding systems on page 4).
This product has been developed by Cell Marque for the Ventana Benchmark platforms, and Cell Marque have their own product line (156R-97/98) of the same Ab, also providing 100% (16/16) sufficient results of which 69% (11/16) were optimal (see Table 1). The majority of assays (14/16) were performed on the Ventana BenchMark platforms, using similar protocol settings as described above.

This was the third NordiQC assessment of CD56. A significant decrease in the pass rate was observed from 81% in Run 37 (2013) to 62% in Run 61 (2021). The most important factors influencing the final result in negative direction were; 1) challenging material circulated to the participants e.g., the neuroendocrine tumour, 2) the use of low sensitive protocol settings e.g., too short HIER, too low concentration of the primary Ab and/or use of a 2-step polymer/multimer detection system and 3) the widely use of mAb clones 123C3, 123C3.D5 or CD564, both as concentrate and RTU formats, providing less successful results on all platforms in general and not only on the Dako Omnis and Ventana BenchMark as seen in previous runs. The reasons for the diverging results observed between the two assessments are currently not known, but most likely caused by the combination of more challenging material being used for the assessment and the reference standard method for establishing the expected results was based on the high affinity and robust rmAb clone MRQ-42 potentially raising the expectations for an optimal CD56 result. However same assessment criteria and relatively similar tissue composition in the three CD56 runs have been applied and the results overall indicate a superior performance of rmAb clone MRQ-42 compared to other clones.

Controls
Tonsil is recommendable as positive and negative tissue control, in which virtually all the NK-cells and CD4/CD8 double positive T-cells must show a strong, predominantly membranous staining reaction. Indicative for an optimal calibrated protocol is, that the fibroblastic reticular cells (meshwork) and the NK-cells should be clearly visible even at very low magnification (2,5x objective). All other subtypes of lymphocytes must be negative.
Appendix is not recommendable as control, as peripheral nerves only display high level of CD56, and limit of detection of CD56 hereby not possible to evaluate and consequently risking misdiagnosis of tumours displaying low level of CD56 expression.

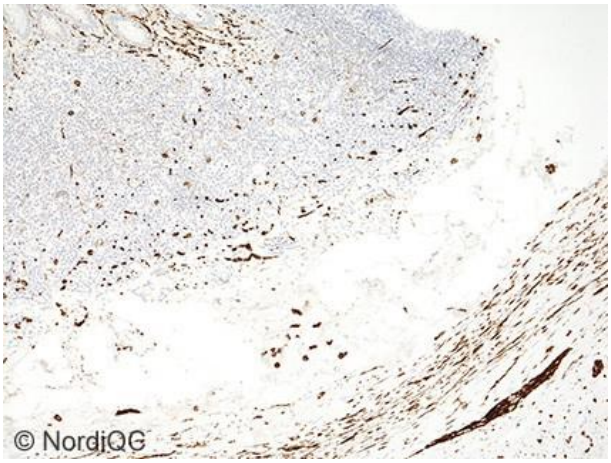


Fig. 1a (x100)
Optimal staining for CD56 of the appendix using the mAb clone 123C3 as a concentrate (1:25), efficient HIER in an alkaline buffer (TRS pH 9, Dako/Agilent) and a 3-step polymer based detection system (EnVision Flex+, Dako/Agilent) on the Omnis - same protocol used in Figs. 2a - 5a. Virtually all peripheral nerves display a strong staining reaction. No background staining is seen and epithelial cells are negative as expected.

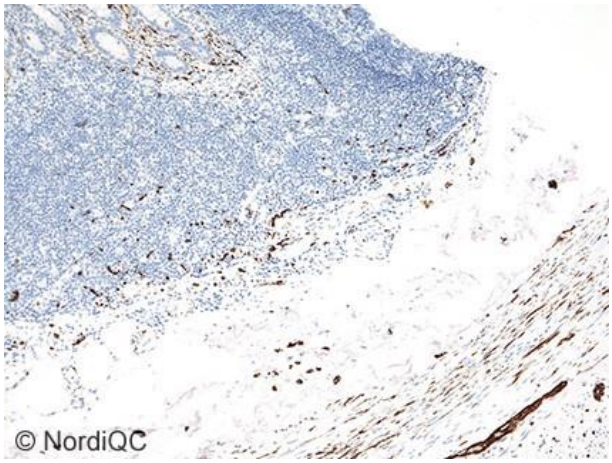


Fig. 1b (x100)
CD56 staining of the appendix based on the mAb clone 123C3 as concentrate (1:25), too short incubation time in primary Ab (20 min.) and the less sensitive detection system EnVision Flex (Dako/Agilent) on the Omnis - same protocol used in Figs. 2b – 5b. Although the staining showed the expected reaction pattern, the protocol overall provided too low analytical sensitivity – see Figs. 2a - 5b. Compare with Fig. 1a - same field.

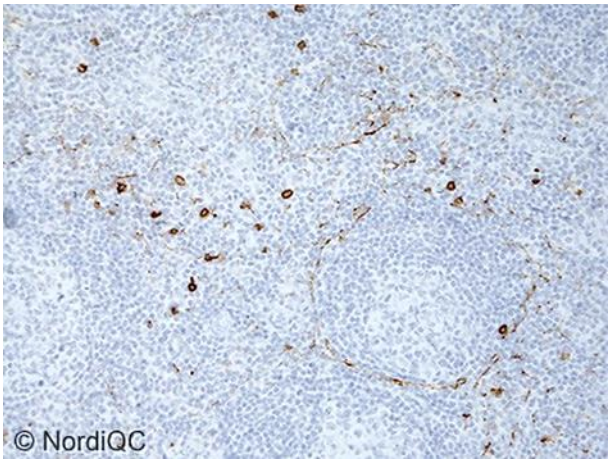


Fig. 2a (x200)
Optimal CD56 staining of the tonsil using same protocol as in Fig. 1a. Virtually all interfollicular NK-cells and CD4/CD8 double positive T-cells display a strong, predominantly membranous staining reaction. The fibroblastic reticular cells (meshwork) show an at least weak staining reaction.

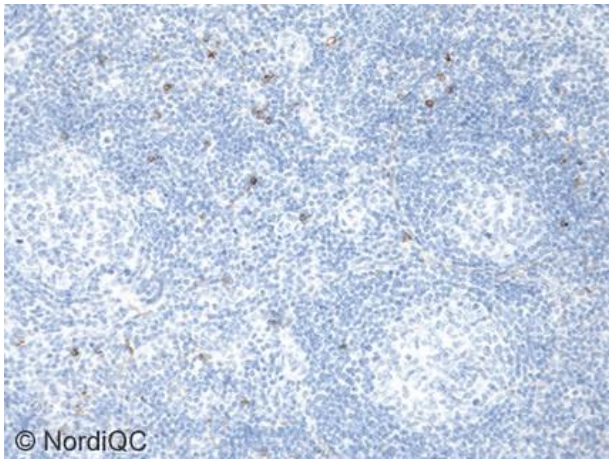


Fig. 2b (x200)
Insufficient CD56 staining of the tonsil using same protocol as in Fig. 1b – same field as in Fig. 2a. The staining intensity of the interfollicular NK-cells and CD4/CD8 double positive T-cells is significantly reduced and the fibroblastic reticular cells are negative or only faintly demonstrated.

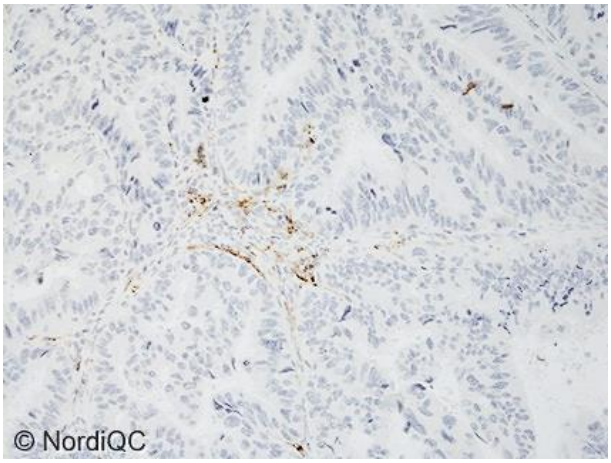


Fig. 3a (x200)
Optimal CD56 staining of the colon adenocarcinoma using same protocol as in Figs. 1a and 2a. All neoplastic cells are negative and only dispersed fibroblastic reticular structures are weakly demonstrated.

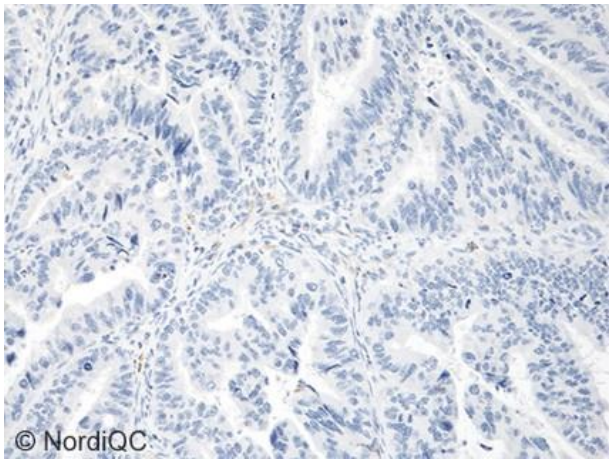


Fig. 3b (x200)
Insufficient CD56 staining of the colon adenocarcinoma, using the same protocol as in Figs. 1b and 2b. The neoplastic cells are as expected negative, but staining intensity of the fibroblastic reticular meshwork is significantly reduced. Compare with Fig. 3a – same field.

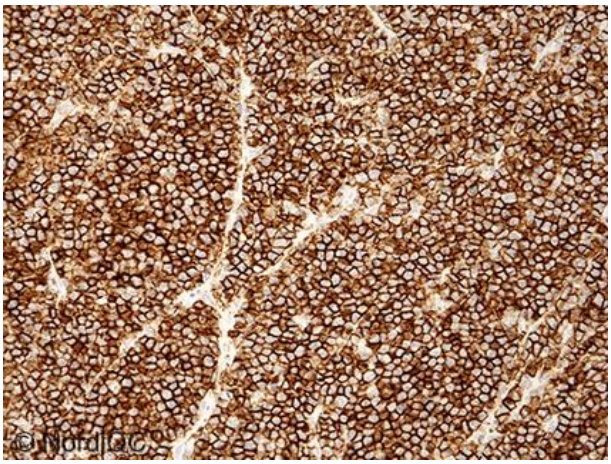


Fig. 4a (x200)
Optimal CD56 staining of the plasmacytoma using same protocol as in Figs. 1a - 3a. All neoplastic cells show a strong and distinct membranous staining reaction.

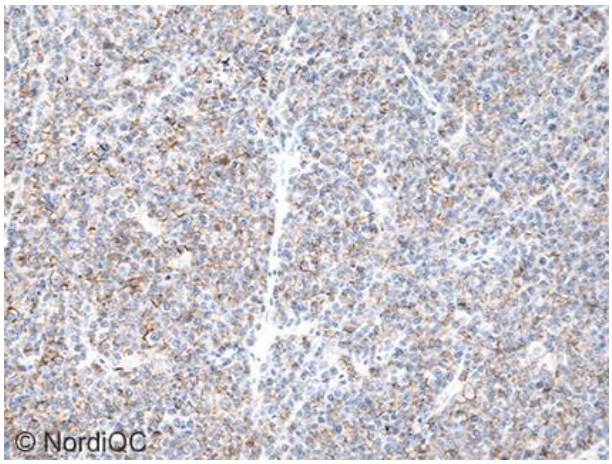


Fig. 4b (x200)
Insufficient CD56 staining of the plasmacytoma using the same protocol as in Figs. 1b - 3b. The staining intensity and proportion of positive cells is reduced, and the majority of the neoplastic cells are only weakly demonstrated. Compare with Fig. 4a – same field.

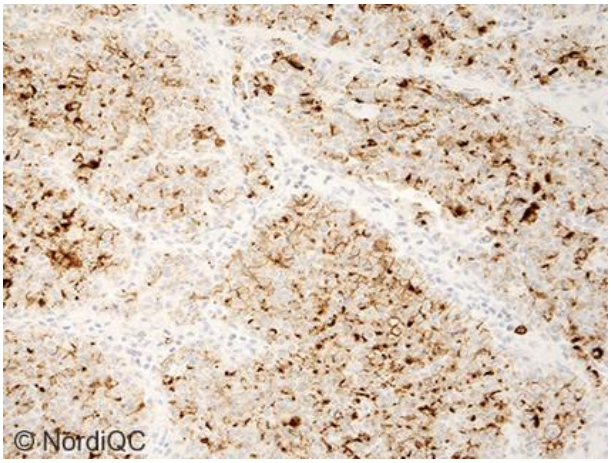


Fig. 5a (x200)
Optimal CD56 staining of the neuroendocrine tumour using same protocol as in Fig. 1a - 4a. The majority of the neoplastic cells display a weak to moderate, predominantly membranous staining reaction.

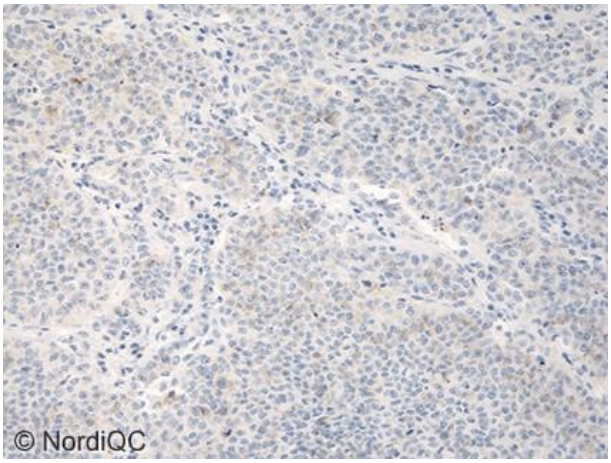


Fig. 5b (x200)
Insufficient CD56 staining of the neuroendocrine tumour using same protocol as in Fig. 1b - 4b. Virtually all neoplastic cells are false negative or only faintly demonstrated. Compare with Fig. 5a – same field.

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