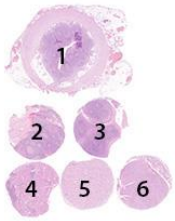
Assessment Run 64 2022
CD56

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. Appendix, 2-3. Tonsil, 4-5. Neuroendocrine carcinoma, 6. Colon Adenocarcinoma.

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).
- An at least weak to moderate, distinct membranous staining reaction of virtually all interfollicular NK-cells and a subset of T cells (CD4/CD8 double positive) in the tonsils.
- A strong, predominantly membranous staining reaction of virtually all neoplastic cells in the neuroendocrine carcinoma (tissue core 4).
- An at least weak to moderate, predominantly membranous staining reaction of the majority of neoplastic cells in the neuroendocrine carcinoma (tissue core 5).
- An at least weak staining reaction of dispersed reticular cells, typically seen in vicinity of secondary follicles in the tonsils and Peyer's patches in the appendix.
- No staining reaction of epithelial cells in appendix (scattered normal neuroendocrine cells of the epithelium might display a weak staining reaction) 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 64	389
Number of laboratories returning slides	364 (94%)

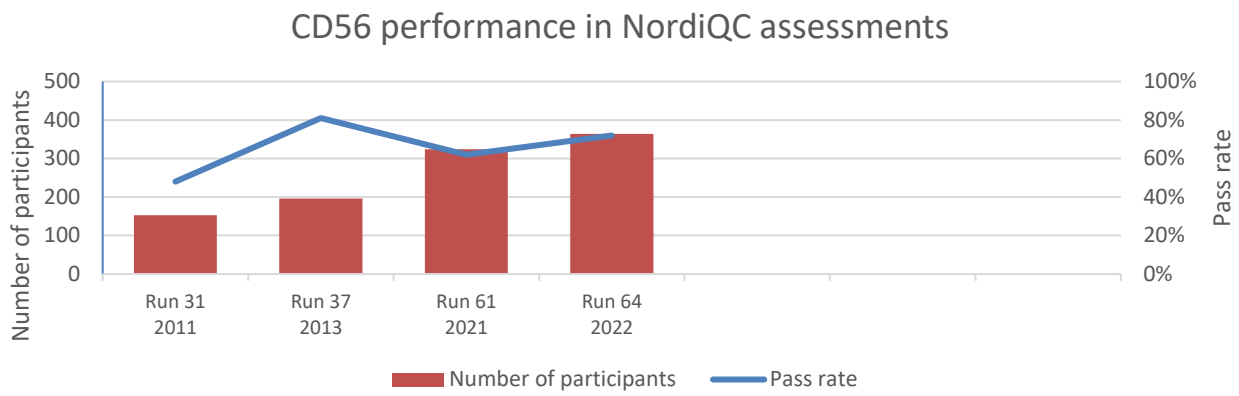
Results
At the date of assessment, 94% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

One laboratory submitted invalid protocol data, and thus, not included in this report. 363 laboratories participated in this assessment and 72% 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 were:
- Less successful performance of the mouse monoclonal Abs (mAb) clone 123C3 and 123C3.D5 on the BenchMark platforms.
- Less successful performance of the Ready-to-use (RTU) format IR/IS628 (Dako/Agilent) on the Omnis.
- Less successful performance of the RTU format PA0191 (Leica Biosystems) on the BOND.
- 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 of acidic buffer.
- Unexplained technical issues.

Performance history
This was the fourth NordiQC assessment of CD56. The pass rate increased marginally compared to previous run 61 (2021) (see Graph 1).

Graph1. **Proportion of sufficient results for CD56 in the four NordiQC runs performed**



Conclusion
The mAb clones 123C3, 123C3.D5, 1B6, CD564, IHC056 and BC56C04, and the rmAb clones MRQ-42 and RCD56 could all be used to obtain an optimal staining result for CD56. Irrespective of the clone applied, efficient HIER in an alkaline buffer, 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. As in previous assessments, the rmAb clone MRQ-42 provided superior performance, both as concentrate and RTU formats, and 94% (189/202) of the results were assessed as sufficient. This clone was very robust and provided optimal results on all main IHC platforms. The mAb clones 123C3 and 123C3.D5 gave inferior results on the BenchMark platforms (Ventana/Roche), and only 13% (3/24) of the protocols based on one of these two clones gave a sufficient result- none being optimal. The mAb clone 123C3, used either as concentrate or RTU format, has in previous runs for CD56 provided poor results on the Omnis (Dako/Agilent) but in this assessment, and applying appropriate protocol settings (high analytical sensitivity) within laboratory developed assays (concentrated antibodies), the pass rate was 93% (14/15) of which 53% (8/15) were optimal. In contrast and using the RTU format IR/IS628 (Dako/Agilent) on the Omnis, also based on the mAb clone 123C3 but tailored for the Autostainer, the performance was less successful providing an overall pass rate of only 13% (3/24) - none being optimal. The RTU format PA0191 (Leica Biosystems) based on the mAb clone CD564 also gave less successful results, mainly related to use of the recommended low pH HIER buffer Bond Epitope Retrieval Solution 1 (BERS1) as none (13/13) of the laboratories using this HIER buffer were able to produce an optimal result and only one laboratory obtained a sufficient result (good).

Tonsil is recommended as positive and negative tissue control: Virtually all the interfollicular NK-cells and CD4/CD8 double positive T-cells must show an at least weak to moderate, distinct membranous staining reaction, while all other subtypes of lymphocytes should be negative.

Table 1. **Antibodies and assessment marks for CD56, Run 64**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **123C3**	28 1 1	Dako/Agilent Genemed Spring Bioscience	11	9	9	1	67%	37%
mAb clone **123C3.D5**	1 2 1 1 1 1 1	Cell Marque Diagnostic Biosystems BioSB Zeta Corporation Immunologic BioLynx Biotech. Unknown	2	1	2	3	38%	25%
mAb clone **CD564**	16	Leica Biosystems	3	5	4	4	50%	19%
mAb clone **1B6**	2	Leica Biosystems	1	0	0	1	-	-
mAb clone **BC56C04**	1	Biocare Medical	1	0	0	0	-	-
mAb clone **IHC056**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **MRQ-42**	63	Cell Marque	44	14	5	0	92%	70%
rmAb clone **RCD56**	2	Zytomed Systems	0	1	0	1	-	-
Unknown	2	Origene	1	0	1	0		
Ready-To-Use antibodies								
mAb clone **123C3, IR628/IS628[3]**	11	Dako/Agilent	1	4	5	1	45%	9%
mAb clone **123C3, IR628/IS628[4]**	41	Dako/Agilent	7	10	18	6	41%	17%
mAb clone **123C3, 790-4465[4]**	13	Ventana/Roche	0	3	10	0	23%	0%
mAb clone **123C3, MAD-000749QD**	3	Master Diagnostica	1	1	1	0	-	-
mAb clone **123C3.D5, 156M-87/88**	2	Cell Marque	0	0	1	1	-	-
mAb clone **C5A2, CCM-0662**	1	Celnovte Biotechnology	0	0	1	0	-	-
mAb clone **CD564, PA0191[3]**	8	Leica Biosystems	0	1	4	3	13%	0%
mAb clone **CD564, PA0191[4]**	17	Leica Biosystems	4	3	10	0	41%	24%
mAb clone **123A8, AMA06**	1	BioGenex	0	0	1	0	-	-
rmAb clone **MRQ-42, 760-4596[4]**	103	Ventana/Roche	73	25	3	2	95%	71%
rmAb clone **MRQ-42, 156R-97/98**	34	Cell Marque	18	13	3	0	91%	53%
rmAb clone **MRQ-42, 8274-C010**	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone **RCD56, RBG/K050**	2	Zytomed Systems	1	1	0	0	-	-
Total	363		171	91	78	23	-	
Proportion			47%	25%	22%	6%	72%	

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 64
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) (10/22)* or TRIS-EDTA/EGTA pH 9 (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, 17 of 19 (89%) 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 Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (2/8) or Cell Conditioning 1 (CC1, Ventana/Roche) (1/4) as retrieval buffer. The mAb was diluted in the range 1:50-1:100. Using these protocol settings, 7 of 11 (63%) laboratories produced a sufficient staining result.

mAb clone **123C3.D5**: Protocols with optimal results were based on HIER in TRS pH 9 (3-in-1) (1/1) or BERS2 (1/1) as retrieval buffer. The mAb was diluted in the range 1:50-1:300. Using these protocol settings, 3 of 4 (75%) laboratories produced a sufficient staining result.

mAb clone **1B6**: One protocol with an optimal result was based on HIER in CC1 as retrieval buffer. The mAb was diluted 1:100 and OptiView with amplification (Ventana/Roche) was used as the detection system.

mAb clone **IHC056**: One protocol with an optimal result was based on HIER in CC1 as retrieval buffer. The mAb was diluted 1:300 and OptiView was used as the detection system.

mAb clone **BC56C04**: One protocol with an optimal result was based on HIER in TRS pH 9 (3-in-1) as retrieval buffer. The mAb was diluted 1:100 and Envision FLEX+ (Dako/Agilent) was used as the detection system.

rmAb clone **MRQ-42**: Protocols with optimal results were based on HIER in CC1 (22/38), TRS High pH (3-in-1) (16/17), BERS2 (5/6) or BERS1 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:500. Using these protocol settings, 50 of 54 (93%) 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**	1/3**	-	8/16 (50%)	-	0/2	-	0/1	0/1
mAb clone **CD564**	-	-	0/1	-	1/3	-	2/8 (25%)	0/3
rmAb clone **MRQ-42**	2/2	-	12/13 (92%)	0/1	20/33 (61%)	-	4/5 (80%)	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), 16-36 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, 66 of 69 (96%) 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, 2 of 2 laboratories obtained an optimal mark.

mAb clone **CD564,** product.no. **PA091**, Leica Biosystems, BOND III/MAX:
Protocols with optimal results were based on HIER in BERS2 (efficient heating time 20-30 min. at 98-100°C), 15-25 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 7 of 9 (78%) laboratories produced a sufficient staining result.

mAb clone **123C3**, product no. **IR/IS628**, Dako/Agilent, Autostainer Link/Classic:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (heating time 20 min. at 97-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8002) as detection system. Using these protocol settings, 10 of 16 (63%) laboratories produced a sufficient staining result.

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**	45% (5/11)	9% (1/11)	92% (12/13)	46% (6/13)
VMS Ultra/XT/GX mAb 123C3 **790-4465**	0/0	0/0	23% (3/13)	0% (0/13)
Leica Bond III/MAX mAb CD564 **PA0191**	13% (1/8)	0% (0/8)	41% (7/17)	24% (4/17)
VMS Ultra/XT/GX **rmAb MRQ-42** **760-4596**	0/0	0/0	95% (97/102)	71% (72/102)

* 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 95% (96/101) 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 reticular cells (in e.g., tonsils) and in the neoplastic cells of the neuroendocrine carcinoma (tissue core 5) was more challenging, requiring an optimally calibrated protocol. The remaining 5% of insufficient results were characterized by excessive background or poor signal-to-noise ratio.

The mAb clones 123C3, CD564 and the rmAb clone MRQ-42 were the most widely used antibodies for demonstration of CD56 and applied by 94% (341/363) of the laboratories (see Table 1).
Used as concentrated format within laboratory developed (LD) assays, the mAb clone 123C3 provided a pass rate of 67% (20/30) of which 37% (11/30) of the protocols gave an optimal result being a significant improvement in performance compared to the latest run 61, giving an overall pass rate of only 46% and 15% being optimal. This development was primarily caused by the use of protocol settings providing a high level analytical sensitivity and in particular observed on the Omnis platform (Dako/Agilent) using settings based on efficient HIER in TRS High for 25-30 min. (97°C), a high working concentration of the primary antibody (range 1:25-1:50) and the sensitive EnVision FLEX+ as detection system.Using these parameters on the Omnis, the pass rate was 93% (14/15) of which 53% (8/15) were optimal. In addition, three laboratories used the mAb clone 123C3 on the Autostainer, with similar protocol settings as on the Omnis, and all (3/3) were giving a sufficient mark with 66% (2/3) being optimal.
The most prevalent feature of an insufficient result, and as described in previous reports, was related to inferior performance of the primary Ab on the Benchmark platforms (Ventana/Roche) as none (5/5) of the protocols based on clone 123C3 provided a sufficient result. 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.

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 (5/5) of the protocols applied on the Benchmark were able to produce a sufficient result. Thus, laboratories applying either the mAb clone 123C3 or 123C3.D5 on the BenchMark platforms, are encouraged to substitute these challenging antibodies. Grouped together and applied both within a LD assay and as RTU format on the BenchMark, only 13% (3/24) were assessed as sufficient – none being optimal.

Used as concentrate, the mAb clone CD564 gave a pass rate of 50% (8/16) of which only 19% (3/16) were assessed as optimal. Optimal results were obtained using efficient HIER in an alkaline buffer, a relative high concentration of the primary Ab (dilution range 1:50-100) and a sensitive 3-step polymer or multimer detection system, BOND Refine or OptiView, respectively. The primary causes of insufficient results were use of protocols with low analytical sensitivity, as inefficient HIER in acidic buffer (e.g., BERS1), too diluted primary ab, too short incubation time in primary Ab or use of a 2-step polymer-based detection system - often applied in combination. The overall pass rate for the mAb clone CD564 in this and the former assessment (Run 61), has been disappointing low and contrasts with the previous assessment (Run 37), in which the mAb clone CD564 provided excellent results, both within LD assays and the RTU system PA0191 (Leica Biosystems). As mentioned in the previous report, the reason for this divergent result is difficult to elucidate upon and currently not known. However, use of the less efficient HIER buffer (BERS1) and more challenging tissue circulated in the two assessment runs e.g., the neuroendocrine carcinoma (tissue core 5) in this run, could be a part of the explanation.

Among the most common antibodies used with a LD assay, the rmAb clone MRQ-42 gave excellent results with an overall pass rate of 92% (58/63) of which 70% (44/63) were optimal (see Table 1). Also, the rmAb clone MRQ-42 provided optimal results on the four main platforms (see Table 2). Although the performance of the concentrated antibody decreased marginally compared to the latest assessment Run 61, the primary Ab is very robust, and several protocol settings could be used to obtain an optimal result. However, the majority of laboratories applied efficient HIER in an alkaline buffer (61/63) and used a 3-step polymer/multimer as detection system (46/63) e.g., Envision FLEX+ or OptiView. 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. For users of the BOND platforms, the detection system BOND Refine acts by nature as a 2-step polymer-system if the host of the primary antibody is produced from a rabbit and only enhances reactions, corresponding to a 3-step polymer detection system, if the primary antibody host is of mouse origin. Therefore, BOND Refine should in principle provide lower sensitivity when protocols are based on rabbit antibodies as e.g., the clone MRQ-42. However, 86% (6/7) of the protocols applying Bond Refine as detection system were assessed as optimal, underlining the robustness of the rmAb clone MRQ-42. For the five protocols based on the rmAb clone MRQ-42 giving an insufficient result no parameters could be identified unraveling the causes for the poor performance. The central protocol settings were fully identical to assays providing optimal results and thus, the five protocols characterized and commented as "unexplained technical issues".

In total, 66% (238/363) of the laboratories used a RTU format for demonstration of CD56 which is in line with the proportion seen in Run 61. Following vendor recommended protocol settings (VRPS), the RTU system IR/IS628 (Dako/Agilent) based on the mAb clone 123C3, tailored for the Autostainer, gave a low pass rate of 45% (5/11) and only 9% (1/11) were giving an optimal mark (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+. In contrast, and using laboratory modified protocol settings (LMPS), the proportion of sufficient and optimal results increased significantly, to 92% (12/13) and 46% (6/13), respectively. The most prevalent feature accounting for this remarkably increase in performance was related to prolonged incubation time in the primary antibody (up to 60 min.).
A significant proportion of participants (n=24) used the RTU format IR/IS628 on the Omnis, most likely due to lack of a RTU alternative to this instrument, providing an overall poor pass rate of 13% (3/24) - none being optimal. This observation, and in accordance with the results obtained in previous runs for CD56, indicate that this RTU product is unable to provide the expected analytical sensitivity on this platform, and thus, laboratories should either use the concentrated format applying the highly sensitive protocol parameters as described above or substitute to an antibody that has proven to give a high proportion of optimal results on the Omnis e.g., mAb clone CAL6. In this context it must be emphasized that, a "direct" transfer of a RTU product developed for the Autostainer to the Omnis platform in general should be avoided or that laboratories at least are obligated to initiate a thorough validation process with focus on purpose and intended use.

The RTU format 760-4465 (Ventana/Roche) also based on the mAb clone 123C3, provided a low proportion of sufficient results (23%, 3/13) of which none were optimal. None of the laboratories used VRPS (see Table 3). Overall, and as observed in previous runs for CD56, the RTU system has consistently

provided inferior results and laboratories are recommended to substitute this product with the RTU format 760-4596 based on the rmAb clone MRQ-42, providing significantly better results (see Table 3).

The Leica RTU system PA0191 (Leica Biosystems) based of mAb clone CD564 gave an overall low pass rate of 32% (8/25) and following VRPS, only 13% (1/8) were sufficient – none being optimal (see Table 3). Applying LMPS, the proportion of sufficient and optimal results increased to 41% (7/17) and 24% (4/17), respectively. The prevalent factor for this improvement was related to substituting the vendor recommended HIER buffer BERS1 (acidic) with BERS2 (alkaline). In total, only 8% (1/13) of the protocols based on HIER in BERS1 gave a sufficient result – none being optimal, whereas protocols based on HIER in BERS2 provided an overall pass rate of 58% (7/12) of which 33% (4/12) were assessed as optimal. In 60% (3/5) of the protocols applying HIER in BERS2, and giving an insufficient mark, the protocol settings were based on too short inefficient HIER time (10-15 min. at 98-100°C). Due to less successful performance during the two latest assessments, the RTU system seems challenging for many laboratories and require that central protocol parameters related to the analytical sensitivity - are optimized. However, this is contradictive with the true nature of a RTU system, and thus, the vendor is highly encouraged to optimize the RTU product, validate and update the recommended protocol settings fulfilling intended use and expected results of the assay.

In this assessment, and among RTU systems from the three major vendors, the RTU system 760-4596 (Ventana/Roche) based on the rmAb MRQ-42 provided the highest proportion of sufficient and optimal results (see Table 3). The pass rate was 95% (97/102) and 71% (72/102) were assessed as optimal. However, none (102/102) of the protocols were based on VRPS (see Table 3). The number of laboratories using this RTU format within a system (BenchMark platforms) has increased significantly from the previous runs to this assessment, from 68 laboratories (Run 61) to 102 (Run 64). The RTU system is very robust, and several protocol settings could be used to obtain an optimal result (see Ready-to-Use antibodies and corresponding systems, Tables 2 and 3). However, and applying all protocol settings, the proportion of optimal results was significantly higher using OptiView compared to UltraView as detection systems, 92% (55/60) and 40% (17/42), respectively. The five protocols assessed as insufficient applied protocol settings similar to assays given a sufficient mark, and thus, categorized and commented as unexplained technical issues.
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 an overall high pass rate of 91% (31/34) of which 53% (18/34) were optimal (see Table 1). The majority of assays (27/34) were performed on the BenchMark platforms (Ventana/Roche), using similar protocol settings as described above. Six laboratories used this format on the Omnis (Dako/Agilent) and all results were assed as sufficient – 50% (3/6) being optimal, typically applying efficient HIER in TRS pH 9 (3-in-1) and EnVision FLEX+ as the detection system

This was the fourth NordiQC assessment of CD56. A minor increase in the pass rate was observed from 62% in Run 61 (2013) to 72% in this Run 64 (2022). The most important factors influencing the final result in negative direction were; 1) challenging material circulated to the participants e.g., the neuroendocrine carcinoma (tissue core 5), 2) the use of low sensitive protocol settings e.g., too short HIER, HIER in low pH buffer, too low concentration of the primary Ab and/or use of a 2-step polymer/multimer detection system, 3) the use of mAb clones 123C3 and 123C3.D5, both as concentrate and RTU formats, on the Ventana BenchMark platforms, 4) use of the RTU format Dako/Agilent IR/IS628 based on the mAb clone 123C3 (tailored for the Autostainer) on the Dako Omnis and 5) used of the RTU system PA0191 based on the mAb CD564 and following recommendations given by the vendor.
Importantly, protocols must stain according to the expected antigen levels, and tonsil is an essential and critical assay performance control assisting to monitor the required level of the analytical sensitivity and specificity of the assay (see below).

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 an at least weak to moderate, distinct membranous staining reaction. All other subtypes of lymphocytes must be negative.
Appendix is not recommendable as positive tissue control, as peripheral nerves are too easy to stain for CD56, and thus, risking misclassification of tumours with low level CD56 expression in the diagnostic work up of tumours of unknown origin.

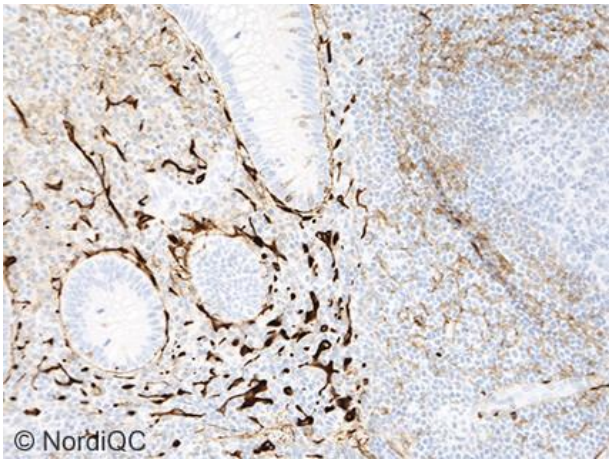


Fig. 1a (x100)
Optimal staining for CD56 of the appendix using the mAb clone CD564 as concentrate (1:50), incubation time in primary Ab for 30 min., efficient HIER in BERS2 (Leica Biosystems) and Bond Refine as the detection system. Staining was performed on the BOND platform - same protocol used in Figs. 2a - 5a. The peripheral nerves display a strong staining reaction, the myofibroblasts and reticular cells of Peyer's patches (meshwork) show a weak to moderate staining intensity.
No background staining is seen and epithelial cells are negative as expected.

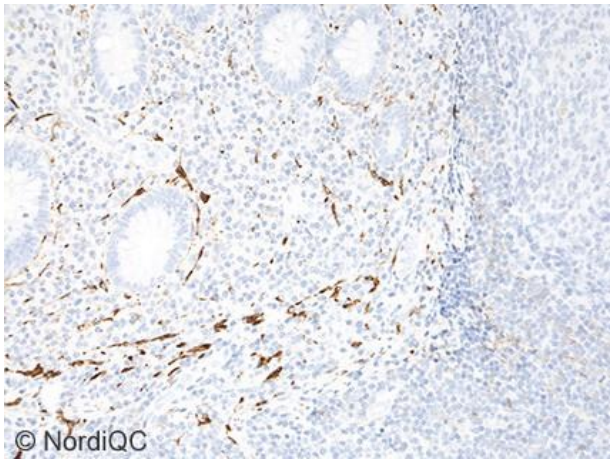


Fig. 1b (x100)
CD56 staining of the appendix also based on the mAb clone CD564 as concentrate (1:50) and on the same platform as in Fig. 1a, but with protocol settings providing too low analytical sensitivity - too short incubation time in primary Ab (15 min.) and HIER in the less efficient buffer BERS1 (Leica Biosystems) - same protocol used in Figs. 2b - 5b. Although the peripheral nerves are stained, the intensity is reduced, and the myofibroblasts and reticular cells are only faintly demonstrated - compare with Figs. 1a - 5b.

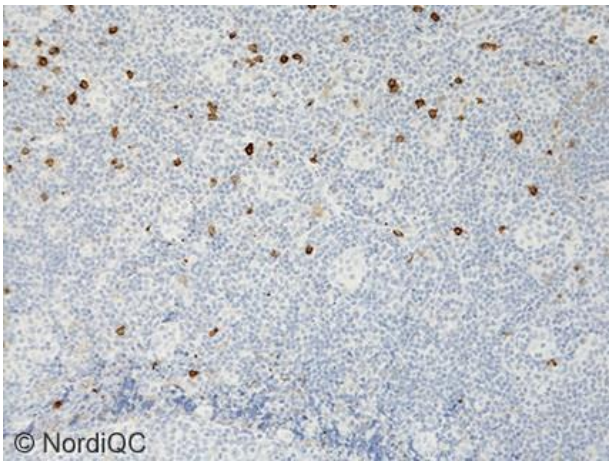


Fig. 2a (x200)
Optimal CD56 staining of the tonsil using same protocol as in Fig. 1a. The interfollicular NK-cells and CD4/CD8 double positive T-cells display a moderate to strong, predominantly membranous staining reaction.

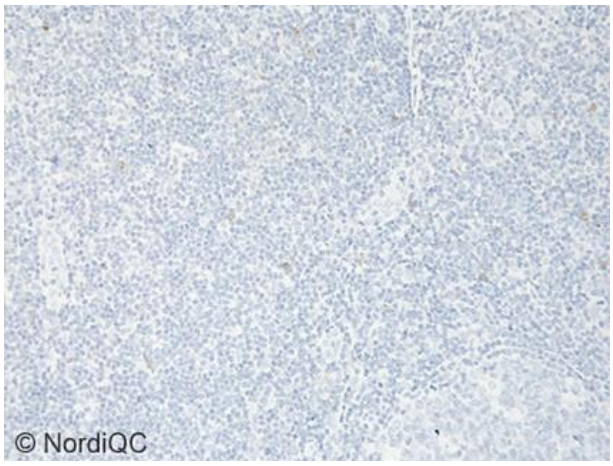


Fig. 2b (x200)
Insufficient CD56 staining of the tonsil using same protocol as in Fig. 1b. The vast majority of the interfollicular NK-cells and CD4/CD8 double positive T-cells are false negative or only faintly demonstrated - compare with Fig. 2a.

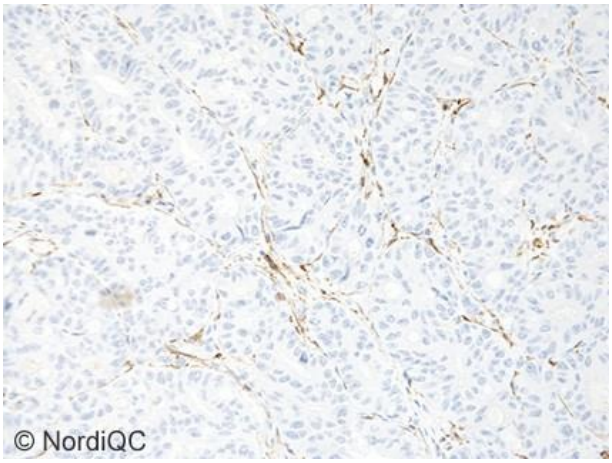


Fig. 3a (x200)
Optimal CD56 staining of the colon adenocarcinoma using same protocol as in Figs. 1a and 2a. All neoplastic cells are negative and dispersed reticular cells are weakly demonstrated.

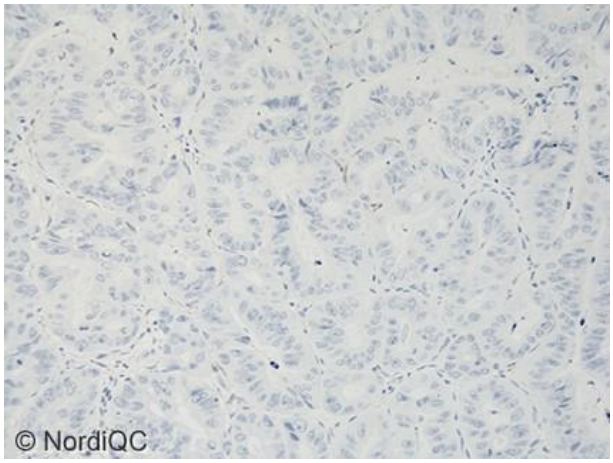


Fig. 3b (x200)
Insufficient CD56 staining of the colon adenocarcinoma, using the same protocol as in Figs. 1b and 2b. The neoplastic cells are as expected negative, but the reticular cells are false negative or only barely visible - compare with Fig. 3a.

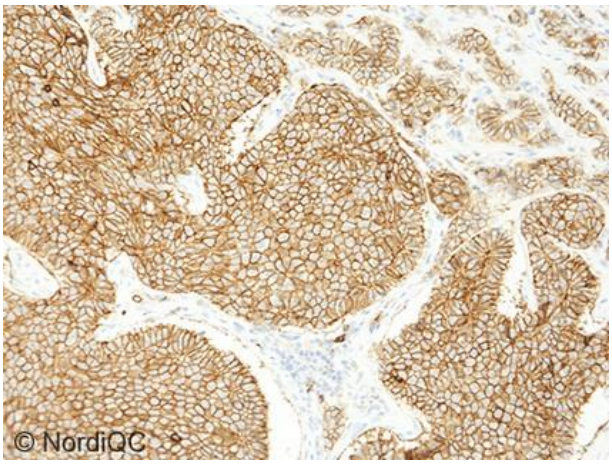


Fig. 4a (x200)
Optimal CD56 staining of the neuroendocrine carcinoma (tissue core 4) using same protocol as in Figs. 1a - 3a. Virtually all neoplastic cells show a strong and distinct membranous staining reaction.

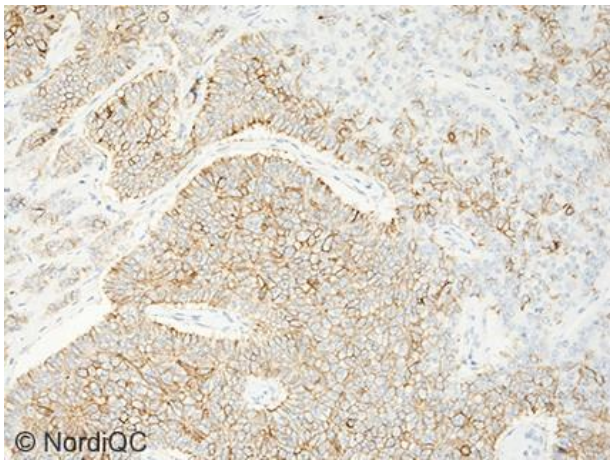


Fig. 4b (x200)
CD56 staining of the neuroendocrine carcinoma (tissue core 4) using the same protocol as in Figs. 1b - 3b. The staining intensity and proportion of positive cells is reduced, and the majority of the neoplastic cells only display a weak to moderate staining reaction - compare with Fig. 4a.

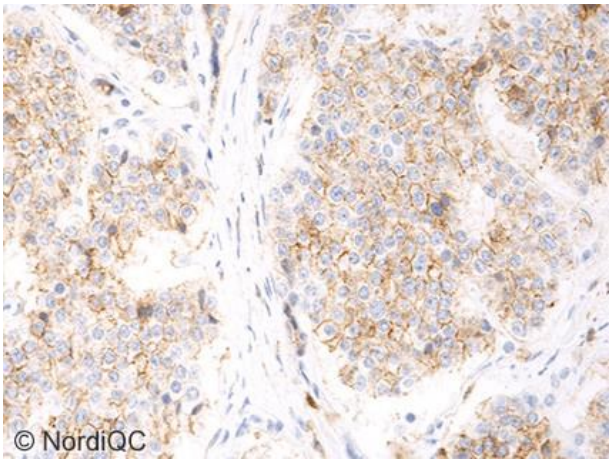


Fig. 5a (x400)
Optimal CD56 staining of the neuroendocrine carcinoma (tissue core 5) using same protocol as in Fig. 1a - 4a. The majority of the neoplastic cells display a weak to moderate, predominantly membranous staining reaction.

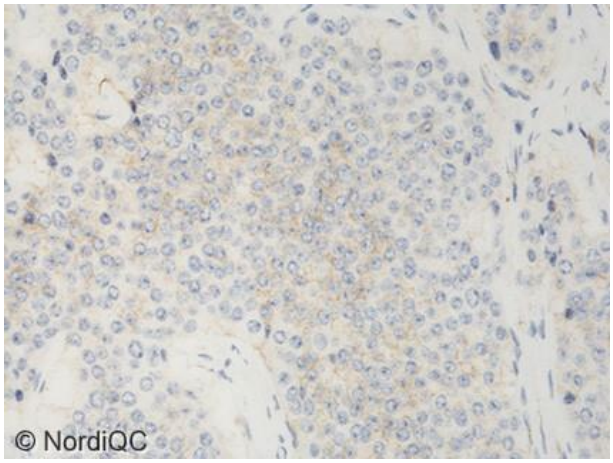


Fig. 5b (x400)
Insufficient CD56 staining of the neuroendocrine carcinoma (tissue core 5) using same protocol as in Fig. 1b - 4b. Virtually all neoplastic cells are false negative or only faintly demonstrated - compare with Fig. 5a.

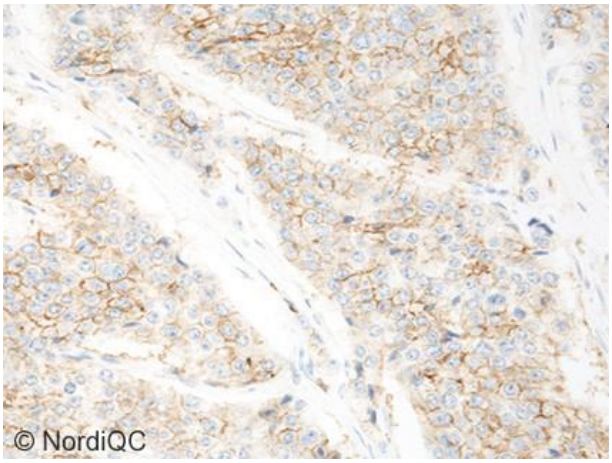


Fig. 6a (x400)
Optimal CD56 staining of the neuroendocrine carcinoma (tissue core 5) using the mAb clone 123C3 as a concentrate (1:50), efficient HIER TRS pH 9 (3-in-1) (Dako/Agilent) and EnVision FLEX+ (Dako/Agilent) as detection system. Staining was performed on the Omnis platform. The protocol provided appropriate analytical sensitivity and the expected reaction pattern – see description in Fig. 5a. Assays based on this clone has in previous assessments been challenged on the Omnis, but for laboratories applying similar protocol settings as described above, the pass rate was 93% of which 53% were optimal.

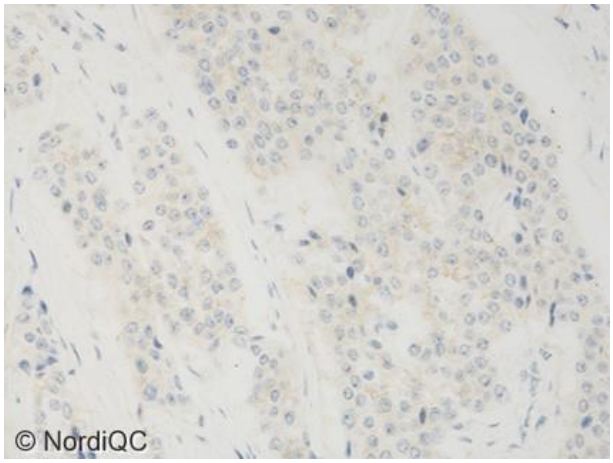


Fig. 6b (x400)
Insufficient CD56 staining of the neuroendocrine carcinoma (tissue core 5) using similar protocol settings as in Fig. 6a, except for the less sensitive detection system EnVision FLEX. Protocols based on the mAb clone 123C3 and used within LD assays (concentrates) on the Omnis, require that all parameters are optimized in relation to intended use and to obtain the required analytical sensitivity. Otherwise, laboratories are risking misdiagnosis of tumours of unknown origin (as in this case) – compare with Fig. 6a.

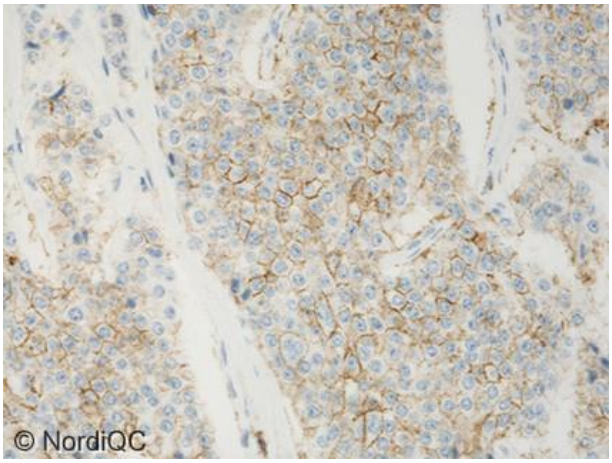


Fig. 7a (x400)
Optimal CD56 staining of the neuroendocrine carcinoma (tissue core 5) using RTU system IR/IS628 (Dako/Agilent) based on the mAb clone 123C3 and applying protocol settings as recommended by vendor on the Autostainer. The protocol provided the expected reaction pattern – see description in Fig. 5a.

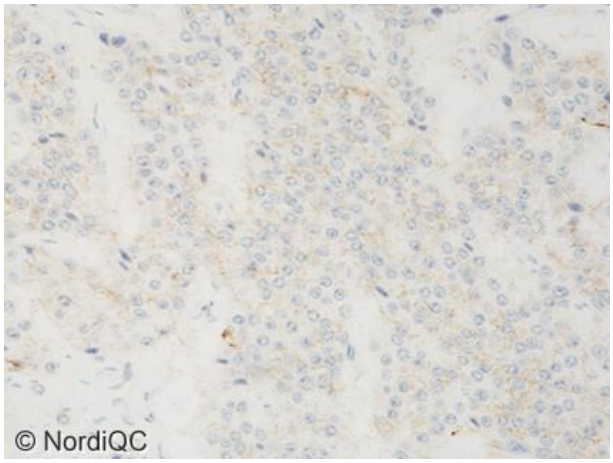


Fig. 7b (x400)
Insufficient CD56 staining of the neuroendocrine carcinoma (tissue core 5) using the same RTU format as in Fig. 7a, but on the Omnis. This RTU product should not be used on this particular platform due to poor performance (see description above), despite using protocol settings that normally provides high analytical sensitivity. Thus, laboratories should substitute this RTU product either with the concentrated format of the mAb clone 123C3 (see Fig. 6a) or the rmAb clone MRQ-42, providing superior performance in this assessment.

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