Assessment Run 37 2013
CD56

Material
The slide to be stained for CD56 comprised:

1. Appendix, 2. & 3. Tonsil, 4. Liver, 5. & 6. Neuroendocrine tumours.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD56 staining as optimal included:

- A strong, predominantly membranous staining of peripheral nerves.
- A strong, predominantly membranous staining of the interfollicular NK-cells and a small subset of T cells (CD4+ & CD8+ double positive)
- A moderate to strong, predominantly membranous staining of virtually all neoplastic cells in the neuroendocrine tumour no. 5
- A moderate to strong, predominantly membranous staining of a subpopulation of neoplastic cells in the neuroendocrine tumour no. 6
- A weak to moderate staining in a proportion of smooth muscle cells.
- A weak to moderate staining in a proportion of endothelial cells in the liver sinusoids.
- A weak to moderate staining in a proportion of fibroblastic reticular cells in tonsils and appendix.
- No staining reaction in the squamous epithelial cells of the tonsil and the hepatocytes in the liver.

200 laboratories participated in this assessment. 4 participants submitted inappropriate antibodies. 81 % of the remaining 196 laboratories achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

Table 1. **Antibodies and assessment marks for CD56, run 37.**

Concentrated Abs	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1B6**	42 1 1	Novocastra/Leica Linaris Vector Lab.	16	17	10	1	75%	77%
mAb clone **123C3**	18 4 2 1	Dako Monosan Invitrogen Spring Bioscience	10	10	3	2	80%	100%
rmAb clone **MRQ-42**	21 1	Cell Marque Immunologic	21	1	0	0	100%	100%
mAb clone **123C3.D5**	18 1	NeoMarkers/Thermo Immunologic	5	6	5	3	58%	100%
mAb clone **CD564**	8 1	Novocastra/Leica Monosan	5	4	0	0	100%	100%
mAb clone **56C04**	2	NeoMarkers/Thermo	1	1	0	0	-	-
rmAb clone **RCD56**	1	Zytomed System	0	0	1	0	-	-
Ready-To-Use Abs:								
mAb clone **123C3, IR628**	34	Dako	16	13	3	2	85%	88%
rmAb clone **MRQ-42 760-4596**	16	Ventana	14	2	0	0	100%	100%
mAb clone **123C3, 790-4465**	9	Ventana	2	1	6	0	33%	-
mAb, clone **CD564, PA0191**	6	Novocastra/Leica	3	3	0	0	100%	100%
mAb, clone **1B6**	4	Novocastra/Leica	0	2	0	2	-	

mAb, clone **123C3.D5, Mon-RTU1049**	1	Monosan	0	1	0	0	-	
mAb clone **BC56C04, PM164**	2	Biocare	0	2	0	0	-	
rmAb clone **MRQ-42, 156R-97**	1	Cell Marque	1	0	0	0	-	
mAb clone **56C04, MAD-000218QD**	1	Master Diagnostica	1	0	0	0	-	
Total	196		95	63	28	10		
Proportion			49%	32%	14%	5%	81%	

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

The following protocol parameters were central to obtain an optimal staining

Concentrated Antibodies
mAb clone **1B6**: Protocols optimal results were all based on heat induced epitope retrieval (HIER) using either Bond Epitope Retrieval Solution 2 (BERS2; Leica) (7/13)*, Cell Conditioning 1 (CC1; Ventana) (3/14), TRS pH 9 (TRS pH 9; Dako) (2/3), TRS pH 9 (3-in-1, Dako) (1/3) or Tris-EDTA/EGTA pH 9 (1/7) as retrieval buffer. The mAb was diluted in the range of 1:10–1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 33 of 43 (77%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **123C3**: Protocols with optimal results were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/3), TRS pH 9 (2/4), TRS pH 9 (3-in-1) (5/8), TRS pH low (3-in-1) (1/1) or BERS2 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:10–1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 15 of 15 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **123C3.D5**: Protocols with optimal results were all based on HIER using either TRS pH 9 (3-in-1) (3/3) or BERS 1 (2/2) as retrieval buffer. The mAb was typically diluted in the range of 1:50–1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 of 5 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **CD564**: Protocols with optimal results were all based on HIER using either CC 1 (4/4) or BERS2 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:20–1:120 depending on the total sensitivity of the protocol employed. Using these protocol settings 8 of 8 (100 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **MRQ-42**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/1), TRS pH 9 (1/1), TRS pH 9 (3-in-1) (1/1) or CC 1 (18/19). The mAb was typically diluted in the range of 1:200–1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 22 of 22 (100%) laboratories produced a sufficient staining (optimal or good).

mAb clone **56C04**: The protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9, 30 min incubation in a 1:100 dilution of the primary Ab and EnVision (K4001) as detection system.

Ready-To-Use Antibodies
mAb clone **123C3** (prod. no. IR628, Dako): Protocols with optimal results were all based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1) and 15-30 min incubation of the primary Ab and a 2- or 3-step polymer system, EnVision Flex (K8000) or Flex+ (K8002) as detection system. Using these protocol settings 28 of 32 (88 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **123C3** (prod. no. 790-4465, Ventana): Protocols with optimal results were all based on HIER in Cell Conditioning 1 (BenchMark instrument) and 24-32 min incubation an incubation time of 24 - 32 min in the primary Ab and a 3-step multimer system OptiView (760-700) as the detection system. Using these protocol settings 2 out of 2 laboratories produced a sufficient staining (optimal or good).

rmAb clone **MRQ-42** (prod. no. 760-4596, Ventana): Protocols with optimal results were all based on HIER in Cell Conditioning 1 (BenchMark instrument), 8-32 min incubation of the primary Ab and a 2 or 3-

step multimer system, UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 16 of 16 (100 %) laboratories produced a sufficient staining (optimal or good).

rmAb clone **MRQ-42** (prod. no. 156R-97, Cell Marque): The protocol with an optimal result was based on HIER using mild Cell Conditioning 1, 16 min incubation of the primary Ab at 36°C and UltraView (760-500) as detection system. Using these protocol settings 1 of 1 laboratory produced an optimal staining.

mAb clone **CD564** (prod. no. PA0191, Novocastra/Leica): Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 1, 15-30 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 4 of 4 (100%) laboratories produced a sufficient staining (optimal or good).

mAb clone **56C04** (prod. no. MAD-000218QD, Master Diagnostica): The protocol with an optimal result was based on HIER in PT-Link using EDTA/EGTA pH8, 10 min incubation of the primary Ab and a 3-step polymer system (Master Diagnostica, MAD-021881QK) as detection system. Using these protocol settings 1 of 1 laboratory produced an optimal staining

The most frequent causes of insufficient staining reactions were:

- Too low concentration of the primary antibody
- Less successful performance of the mAb clones 123C3 and 123C3.D5 on the Ventana BenchMark platform.

In concordance with the previous NordiQC assessment of CD56 (run 31, 2011), the prominent feature of an insufficient staining was a too weak or false negative staining of structures expected to be demonstrated. The majority of the laboratories were able to demonstrate CD56 in high antigen expressing structures as peripheral nerves (Fig. 1a and 1b), whereas NK-cells (Fig. 2a and 2b) and the neoplastic cells of the 2 neuroendocrine carcinomas (tumours 5 and 6) (Fig. 3a and 3b) were more challenging and required an optimally calibrated protocol.

In this assessment, all participants used HIER. 89 of 95 protocols with optimal results used an alkaline HIER buffer. Optimal staining could be produced with all clones except for mAb clone BC56C04 and rmAb clone RCD56. The most widely used Abs were the mAb clones 123C3, 1B6, 123C3.D5, CD564 and the rmAb clone MRQ-42. The performance of the rmAb clone MRQ-42 and mAb CD564 were excellent. Both clones produced pass rates (optimal or good) of 100% for both concentrates and RTU's. High pass rates were also seen with mAb clone 123C3 (80% for concentrate) and mAb 1B6 (75% for concentrate). In contrast only 58% using mAb 123C3.D5 (concentrate) passed. One reason was less successful performance of mAb clone 123C3.D5 on the Ventana BenchMark platform where only 3 of 7 laboratories (43%) achieved sufficient marks compared to non-Ventana platforms where 8 of 12 laboratories (67%) achieved a sufficient mark with this clone. The performance of mAb clone 123C3 was likewise influenced by the stainer platform used. Only 3 of 9 laboratories (33%) using the Ventana RTU of mAb clone 123C3 on the BenchMark platform achieved sufficient marks in contrast to 29 out of 33 laboratories (88%) using Dako RTU on non-Ventana platform stainers. The reasons for these discrepancies are currently not known. Other clones performed well on the Ventana BenchMark platform. In the current assessment 36 laboratories used rmAb clone MRQ-42 (concentrate or RTU) on the Ventana BenchMark platform and the pass rate was 100% with a very high proportion of optimal marks (92%). A similar 100% pass rate was seen in 7 laboratories using mAb clone CD564 (concentrate) on the Ventana BenchMark platform (4/7 achieved optimal marks).

Controls
Tonsil is recommendable as a reliable positive control, in which virtually all the NK-cells and CD4 and CD8 double positive T-cells must show a strong, predominantly membranous staining reaction. The NK-cells should be clearly visible even at very low magnification (2,5x objective) (Fig. 2a and 2b).
No staining of other subtypes of lymphocytes must be seen Because of the high content of CD56, peripheral nerves alone should not be used as positive controls (Fig. 1a and 1b).

Effect of external quality assessment
This was the second assessment of CD56 general, marked improvement in the proportion of sufficient results was seen in the current run. 81% were sufficient in the current run compared to 48 % in run 31, 2011 – see table 2.

Table 2. **Proportion of sufficient results for CD56 in the two NordiQC runs performed.**

	Run 31 2011	Run 37 2013
Participants, n=	153	196
Sufficient results	48%	81%

The significant improvement of the pass rate for CD56 could by be influenced by many factorsOne important cause seems to be the introduction of rmAb clone MRQ-42 on the Ventana BenchMark platform at the expense of mAb clone 123C3.D5 and mAb clone 123C3. Data shows that 19 laboratories of 31 (61%) using mAb clone 123C3.D5 or mAb 123C3 at the Ventana BenchMark platform in run 31 changed to rmAb MRQ-42 in run 37. All 19 labs improved their insufficient stainings and subsequently received sufficient marks (optimal or good).

Conclusion

The mAbs clones 123C3, 1B6, CD564, 123C3.D5 and 56C04 and the rmAb clone MRQ-42 can all be used to obtain an optimal staining for CD56. The performances of some Abs seem to be influenced by the stainer platform. On the Ventana BenchMark platform the rmAb clone MRQ-42 and the mAb clone CD564 should be preferred, both giving a very high proportion of optimal results (92% optimal with MRQ-42 and 57% optimal with CD564). On non-Ventana platforms, choice of clone is not as critical. Tonsil is a recommended positive control: Virtually all the interfollicular NK-cells and CD4 and CD8 double positive T-cells must show a strong, predominantly membranous staining reaction.

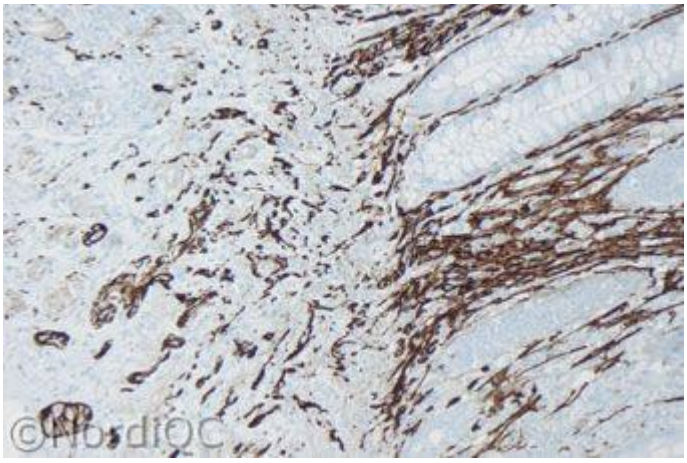

Fig. 1a
Optimal CD56 staining of the appendix using the rmAb clone MRQ-42 (Ventana RTU) optimally calibrated and with HIER in an alkaline buffer and performed on the BenchMark Ultra, Ventana. A strong staining reaction is seen in virtually all the peripheral nerves. No background staining is seen.

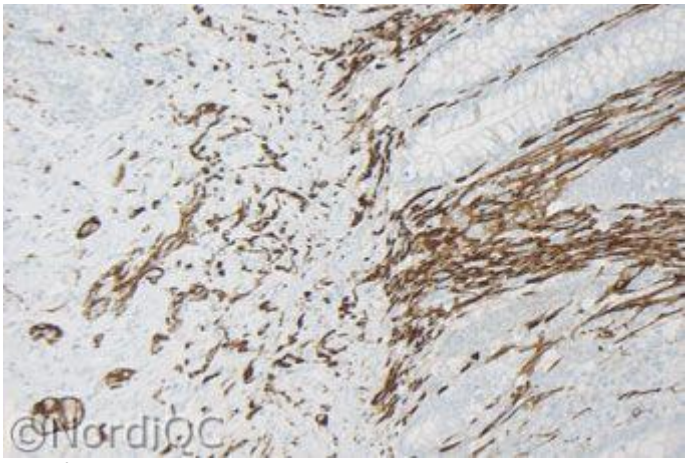

Fig. 1b
CD56 staining using an insufficient protocol based on the mAb clone 123C3 (Ventana RTU) with HIER in an alkaline buffer and performed on the BenchMark Ultra, Ventana. A moderate staining reaction is seen in the majority of the peripheral nerves – same field as in Fig. 1a.
Also compare with Figs. 2b and 3b – same protocol.

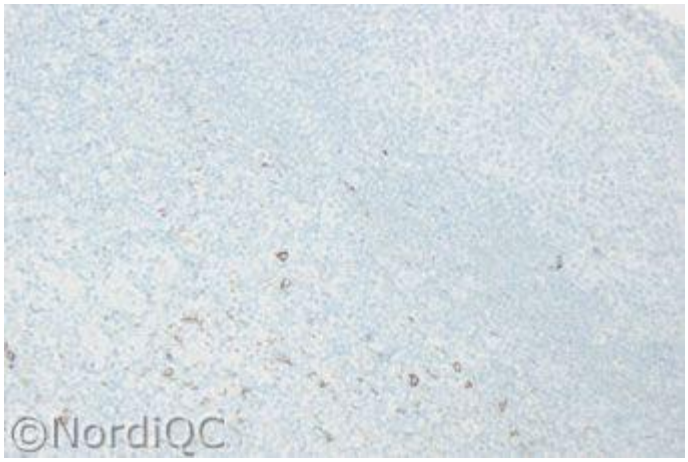

Fig. 2a
Optimal CD56 staining of the tonsil using same protocol as in Fig. 1a. A strong predominantly membranous staining reaction is seen in virtually all the interfollicular NK-cells. Even at low magnification (2,5x objective) the NK-cells are easily identified.

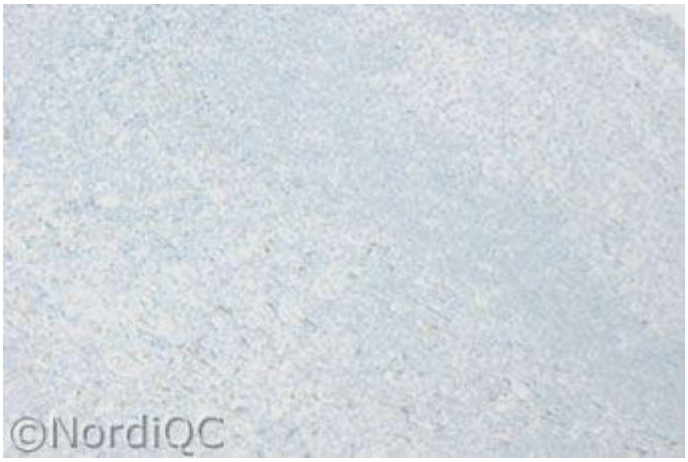

Fig. 2b
Fig. 2b. Insufficient CD56 staining using same protocol as in Fig. 1b. Only a few, faintly positive interfollicular NK-cells are seen - same field as in Fig. 2a.

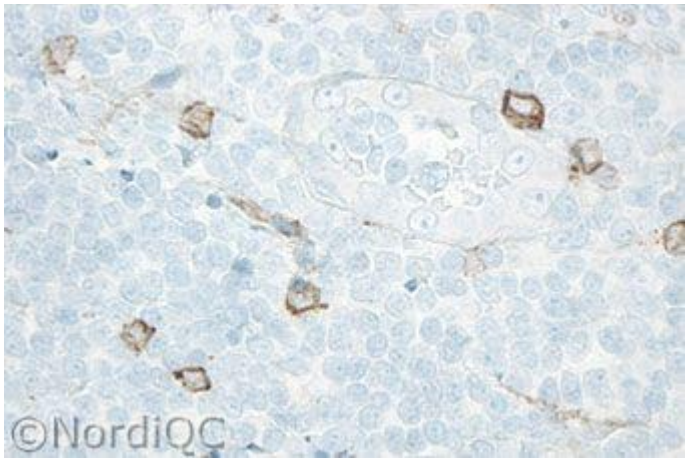

Fig. 3a
High magnification (200x objective) of the tonsil shown in Fig. 2a.
The CD56 positive lymphocytes show a distinct membranous staining reaction. No background staining is seen.

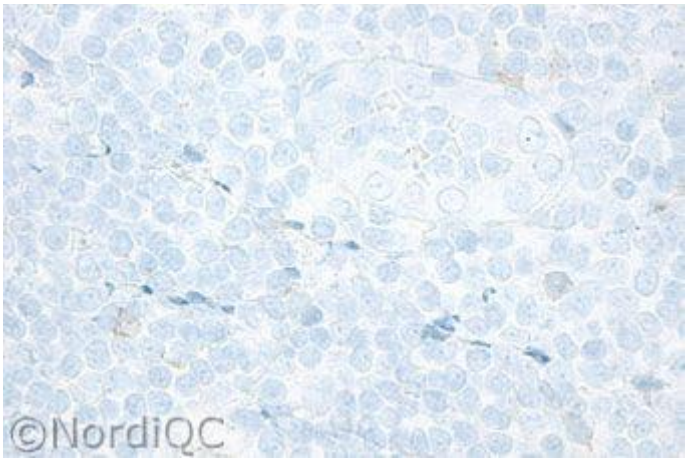

Fig. 3b
High magnification (200x objective) of the tonsil shown in Fig. 3b.
The proportion and the intensity of the CD56 positive lymphocytes is significantly reduced.

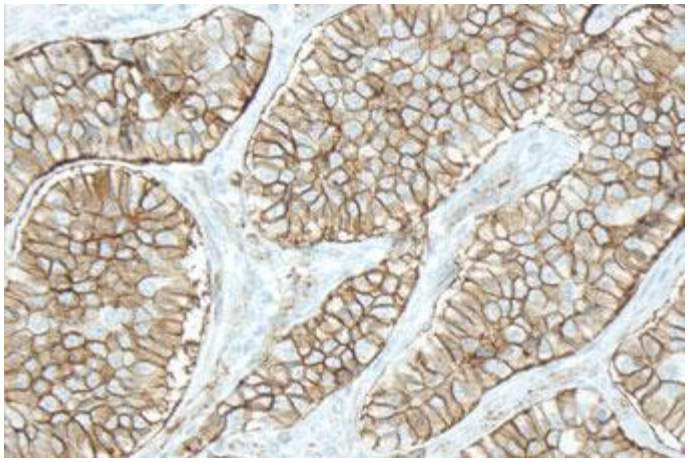

Fig. 4a
Optimal CD56 staining of the neuroendocrine tumour (5) using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a strong and distinct membranous staining reaction.

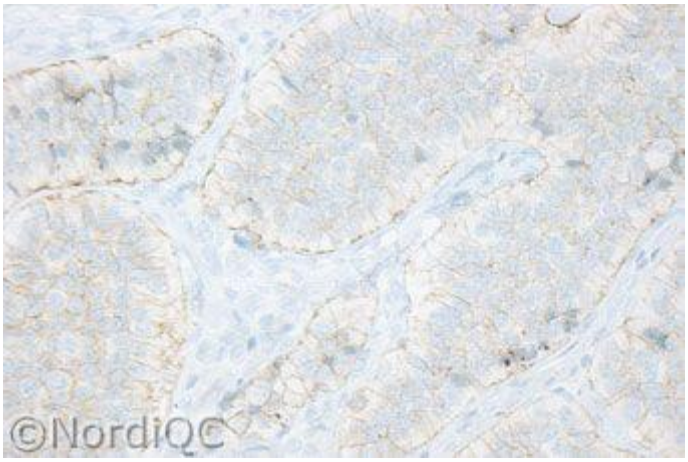

Fig. 4b
Insufficient CD56 staining of the neuroendocrine tumour (5) using same protocol as in Figs. 1b - 3b. Only scattered neoplastic cells show a weak or equivocal staining reaction – same field as in Fig. 4a.

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