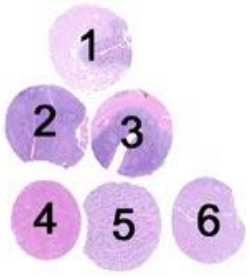
Assessment Run 31 2011
CD56



The slide to be stained for CD56 comprised:

1. Appendix, 2-3. Tonsil, 4. Liver, 5. Caecum carcinoid, 6. Pancreas neuroendocrine carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD56 staining as optimal included:

- A strong, predominantly membranous staining of the peripheral nerves in the appendix.
- A moderate to strong, predominantly membranous staining of virtually all the interfollicular NK-cells and a small subset of T cells (CD4+/CD8+) in the tonsils.
- A weak to moderate staining in scattered fibroblastic reticular cells in the tonsils and appendix.
- A moderate to strong, predominantly membranous staining of virtually all the neoplastic cells of the carcinoid and the neuroendocrine carcinoma.
- No staining in the squamous epithelial cells of the tonsil and the hepatocytes of the liver.

153 laboratories participated in this assessment. 48 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD56, run 31**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1B6**	35	Leica/Novocastra	3	11	12	9	40 %	75 %
mAb clone **123C3**	12 5 5 2 1 1 1 1 1	Dako Monosan Invitrogen/Zymed Zytomed BioCare BioSite Meridan Nova Kemi Spring Bioscience In house	6	8	9	7	47 %*	100 %
mAb clone **123C3.D5**	28 1	NeoMarkers/Thermo ID Labs	2	10	8	9	41 %*	-
mAb clone **CD564**	9	Leica/Novocastra	1	2	2	4	33 %	-
rmAb clone **MRQ-42**	4	Cell Marque	3	1	0	0	-	-
mAb clone **56C04**	3 1	NeoMarkers/Thermo Master Diagnóstica	1	2	1	0	-	-
mAb clone **BC56C04**	1	Biocare	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **123C3, IR628**	17	Dako	9	8	0	0	100 %	100 %
mAb, clone **123C3.D5, 760-2625	11	Ventana/Cell Marque	0	0	1	10	0 %*	-
mAb clone **123C3, 790-4465**	5	Ventana	0	1	2	2	20 %*	-
mAb, clone **CD564, PA0191**	3	Leica/Novocastra	1	2	0	0	-	-
mAb, clone **123C3.D5, MS-204-R7**	2	NeoMarkers/Thermo	0	0	2	0	-	-
mAb, clone**123C3.D5,**	1	Monosan	0	0	1	0	-	-

Mon-RTU1049								
mAb clone **BC56C04, PM164**	1	Biocare	0	1	0	0	-	-
rmAb clone **MRQ-42, 156R-97**	1	Cell Marque	1	0	0	0	-	-
Total	153		27	46	38	42	-	-
Proportion			18 %	30 %	25 %	27 %	48 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
*) Low scores partly due to poor performance on Ventana platforms
**) Product discontinued 2010

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **1B6**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1, Dako) (3/4)*. The mAb was diluted in the range of 1:25–1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 4 laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **123C3**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/3), TRS pH 9 (Dako) (2/6), TRS pH 9 (3-in-1, Dako) (2/3) or Citrate pH 6 (1/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:10– 1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 9 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **123C3.D5**: The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (3-in-1) and an incubation time of 20-30 min in the primary Ab, diluted 1:50 and using a 2-step polymer system, EnVision Flex (K8000, Dako) as the detection system. Using these protocol settings both laboratories produced an optimal staining.

mAb clone **CD564**: The protocol giving an optimal result was based on HIER using standard Cell Conditioning 1 (CC1;Ventana), an incubation time of 32 min in the primary Ab diluted 1:20 and UltraView (760-500, Ventana) as the detection system.

rmAb clone **MRQ-42**: The protocols giving an optimal result were all based on HIER using mild or standard CC1. The mAb was typically diluted in the range of 1:500 – 1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 4 laboratories produced a sufficient staining.

mAb clone **56C04**: The protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9, an incubation time of 30 min in the primary Ab, diluted 1:100 and EnVision+ (K4001, Dako) as the detection system. Using similar protocol settings 2 out of 3 laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **123C3** (prod. no. IR628, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9, TRS pH 9 (3-in-1) or Tris-EDTA/EGTA pH 9, an incubation time of 20 min in the primary Ab and a 2 or 3-step polymer system, EnVision Flex (K8000, Dako) or Flex+ (K8002, Dako) as the detection system. Using these protocol settings all of 17 (100 %) laboratories produced a sufficient staining.

mAb clone **CD564** (prod. no. PA0191, Leica/Novocastra): The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 2, an incubation time of 25 min in the primary Ab and Bond Polymer Refine Detection (DS9800, Leica) as the detection system.

rmAb clone **MRQ-42** (prod. no. 156R-97, Cell Marque): The protocol giving an optimal result was based on HIER using mild CC1, an incubation time of 16 min of the primary Ab at 36°C and UltraView (760-500, Ventana) as the detection system.

The most frequent causes of insufficient stainings 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 this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative reaction of the cells expected to be demonstrated. The majority of the laboratories were able to demonstrate CD56 in high antigen expressing structures such as peripheral nerves (Fig. 1a and 1b), whereas the low expressing NK-cells (Fig. 2a and 2b), neoplastic cells of the carcinoid and in particular the neuroendocrine carcinoma (Fig. 3a and 3b) was more challenging and required an optimally calibrated protocol.

In this assessment all participants used HIER. 26 protocols out of 27 producing optimal results were based on the use of an alkaline HIER buffer. In spite of the low number of optimal results, an optimal staining could be produced with all clones applied in this assessment except for the mAb clone BC56C04. The most widely used Abs were the mAb clones 1B6, 123C3.D5 and 123C3 applied as a concentrate with an in-house calibrated system. All the 3 clones showed a very low pass rate ranging from 40% to 47%. The pass rates of the Abs were highly influenced by the stainer platform used: On the Ventana BenchMark platform a less successful performance was seen with both the concentrates and the Ready-To-Use (RTU) formats of the mAbs clones 123C.D5 and 123C3. No Ventana BenchMark users were able to produce an optimal staining with these 2 clones. With clone 123C3.D5 (RTU and concentrates) Ventana users had a pass rate of 4% (1 laboratory out of 23). For clone 123C3 (RTU and concentrates) the pass rate was 13% (2 laboratories out of 15). For non-Ventana platform users the overall pass rates for these 2 clones were significantly higher. Non-Ventana platform users had a pass rate of 52% (11 laboratories of 21) for clone 123C3.D5 (RTU and concentrates). For clone 123C3 (RTU and concentrates) that figure was 81% (30 laboratories of 37). The reason for these discrepancies is currently not known.
Although the numbers are small, the new rmAb clone MRQ-42 from Cell Marque looks promising, especially for Ventana users: All of 5 laboratories produced a sufficient staining with the rmAb clone MRQ-42 on the Ventana BenchMark platform.

In contrast to the relatively low pass rates for the concentrates in general (47%), the RTU format of the mAb clone 123C3 from Dako showed a pass rate of 100% (17 laboratories). The RTU format of the mAb clone CD564 from Leica/Novocastra also had a higher pass rate than the concentrate.

Tonsil seems to be a recommendable positive control: The NK-cells must show a strong, predominantly membranous staining reaction and should be clearly visible even at very low magnification (2,5x objective). Because of the high content of CD56, peripheral nerves alone should not be used as positive controls.

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 the Abs seem to be influenced by the stainer platform. On non-Ventana platforms the mAb clone 123C3 both as concentrate and as RTU format gave the highest proportion of optimal results. On the Ventana BenchMark platform the rmAb clone MRQ-42 gave the highest proportion of optimal results.
Tonsil is a recommended positive control: The interfollicular NK-cells must show a strong, predominantly membranous staining reaction.

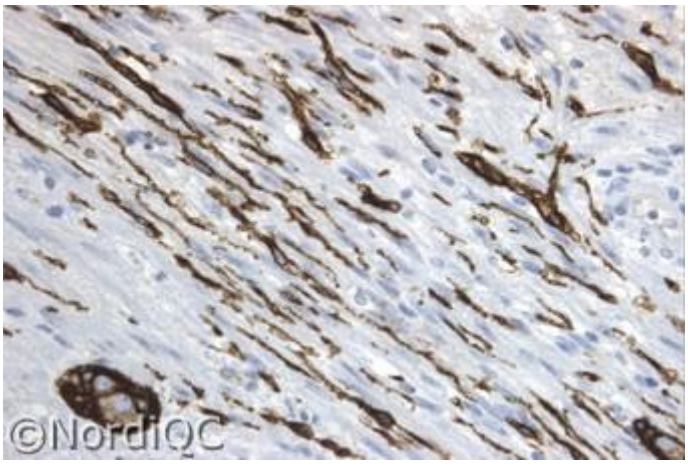


Fig. 1a
Optimal CD56 staining of the appendix using the mAb clone 1B6 optimally calibrated and with HIER in an alkaline buffer. A strong staining reaction is seen in virtually all the peripheral nerves. No background staining is seen.

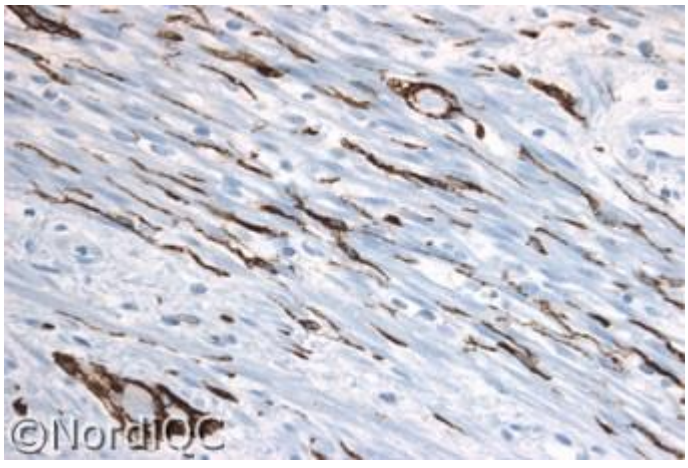


Fig. 1b
CD56 staining using an insufficient protocol based on the mAb clone 123C3 with HIER in an alkaline buffer and performed on the BenchMark Ultra, Ventana.
A moderate staining reaction is seen in the majority of the peripheral nerves – same field as in Fig. 1a. Also compare with Figs. 2b and 3b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

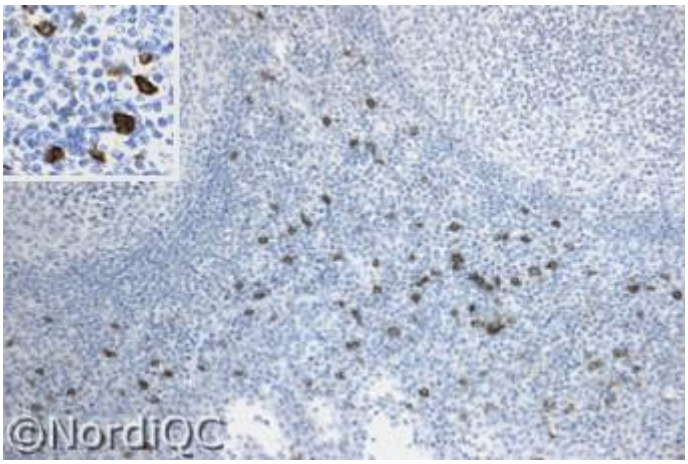


Fig. 2a
Optimal CD56 staining of the tonsil using same protocol as in Fig. 1a. A strong predominantly membranous staining reaction is seen in virtually all the interfollicular NK-cells. Even at low magnification (2,5x objective) the NK-cells are easily identified. Insert: high magnification (x200).

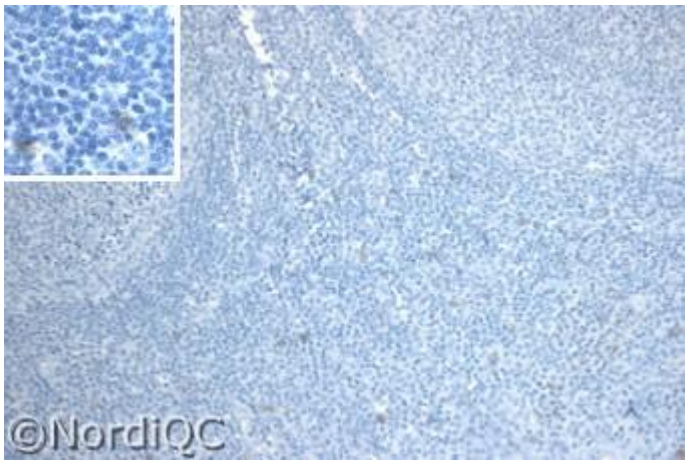


Fig. 2b
Insufficient CD56 staining using same protocol as in Fig. 1b. Only a few, faintly positive interfollicular NK-cells are seen - same field as in Fig. 2a. Insert: high magnification (x200).

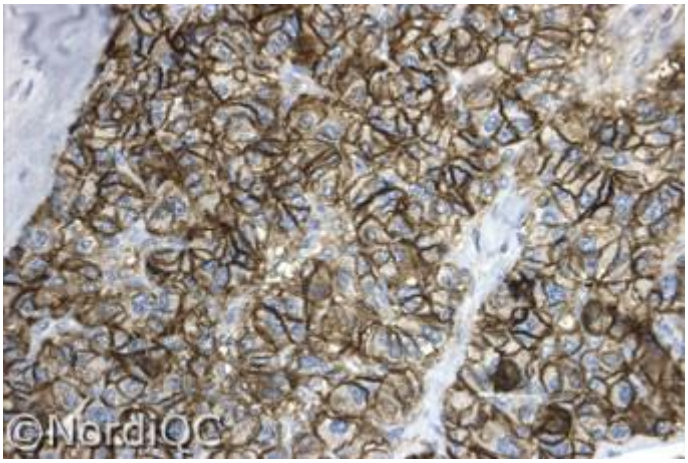


Fig. 3a
Optimal CD56 staining of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct membranous staining reaction.

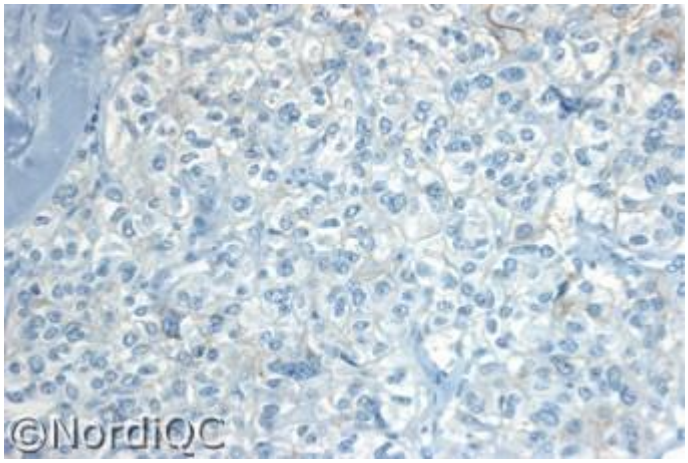


Fig. 3b
Insufficient CD56 staining of the pancreatic neuroendocrine carcinoma using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak or equivocal staining reaction – same field as in Fig. 3a.

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