Assessment Run 37 2013

Synaptophysin (SYP)

Material
The slide to be stained for SYP comprised:

1. Colon, 2. Small cell lung carcinoma (SCLC), 3. Adrenal gland, 4. Colon adenocarcinoma, 5. Intestinal carcinoid, 6. Pancreas neuroendocrine tumour

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a SYP staining as optimal included:

- A moderate to strong, distinct cytoplasmic staining reaction of neuroendocrine cells, ganglion cells and the axons of the nerve plexus in the colon
- A weak to moderate staining of the majority of goblet cells in the colon mucosa
- A moderate to strong, distinct cytoplasmic, dot-like reaction in the majority of cortical epithelial cells of the adrenal gland.
- At least moderate, distinct cytoplasmic, staining reaction in the majority of neoplastic cells of the small cell lung carcinoma, the intestinal carcinoid and the pancreas neuroendocrine carcinoma.
- No staining of the neoplastic cells of the colon adenocarcinoma.

213 laboratories participated in this assessment. 123 (58 %) achieved a sufficient mark. Table 1 summarizes the antibodies (Abs) used and assessment marks.

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

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **27G12**	82 3 1	Leica/Novocastra Monosan Vector	19	45	18	4	74 %	78 %
mAb clone **Snp88**	14	Biogenex	1	3	8	2	29 %	-
mAb **SY38**	10	Dako	0	0	5	5	-	-
rmAb **EP158**	1	Epitomics	0	0	1	0	-	-
rmAb **MRQ-40**	3	Cell Marque	2	1	0	0	-	-
rmAb **SP11**	7 4 1 1 1	Thermo/Neomarkers Spring Abcam Diagnostic Biosystems Maxin	5	3	6	0	57 %	86 %
pAb **A0010**	4	Dako	0	1	1	2	-	-
pAb **RB-1461**	2	Thermo/Neomarkers	0	1	1	0	-	-
pAb **336A-76**	1	Cell Marque	0	1	0	0	-	-
pAb **18-0130**	1	Invitrogen/Zymed	0	1	0	0	-	-
Ready-To-Use Antibodies								
mAb clone **27G12 PA0299**	7	Leica/Novocastra	0	5	2	0	71 %	-
mAb clone **27G12 PM371**	1	Biocare	0	0	0	1	-	-
mAb clone **27G12 MONX10781**	1	Monosan	0	0	1	0	-	-
mAb clone **Snp88 AM363**	1	Biogenex	0	0	1	0	-	-
mAb clone **SY38**	22	Dako	0	0	6	16	-	-

IS/IR776								
rmAb clone **MRQ-40 760-4595**	16	Ventana/Cell Marque	6	7	3	0	81 %	92 %
rmAb clone **MRQ-40 336R**	3	Cell Marque	0	3	0	0	-	-
rmAb clone **SP11 790-4407**	23	Ventana	6	11	6	0	74 %	76 %
rmAb clone **SP11 MAD-000313QD**	1	Master Diagnostica	0	0	1	0	-	-
rmAb clone **SP11 ZA0506**	1	Zhongshan	0	1	0	0	-	-
Unknown	1	Unknown	1	0	0	0	-	-
Total	213		40	83	60	30	-	
Proportion			19 %	39 %	28 %	14 %	58 %	

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

Detailed analysis of SYP, Run 37
The following protocol parameters were central to obtain an optimal staining:

Concentrated Abs
mAb clone **27G12**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9; Dako) (7/14)*, TRS pH 9 (Dako) 6/10, Cell Conditioning 1 (CC1; Ventana) (1/30), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (2/12), Diva Decloaker pH 6.2 (Biocare) (1/2) or Tris-EDTA/EGTA pH 9 (2/10) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 49 of 63 (78 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **Snp88**: The protocol with an optimal result was based on HIER using TRS pH 9 (Dako) (1/2) as retrieval buffer. The mAb was diluted 1:50.

rmAb clone **MRQ-40**: Protocols with optimal results were all based on HIER using either TRS pH 9, 3-in-1 (Dako) (1/1) or CC1 (Ventana) (1/2) 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 2 of 2 (100 %) laboratories produced an optimal staining.

rmAb clone **SP11**: Protocols with optimal results were all based on HIER using either TRS pH 9, 3-in-1 (Dako) (1/1), CC1 (Ventana) (3/12) or Tris-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 6 of 7 (86 %) laboratories produced a sufficient staining (optimal or good).

Table 2 summarizes the overall proportion of optimal staining results using the three most frequently used concentrated abs and IHC stainer platforms.

Table 2. **Optimal results for SYP using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
Buffer	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 **27G12**	54 % 13/24**	0 % 0/3	4 % 1/26	0 % 0/3	18 % 2/11	0 % 0/3
mAb clone **Snp88**	100 % 1/1	-	0 % 0/3	-	0 % 0/2	-
rmAb **SP11**	50 % 1/2	-	40 % 2/5	-	-	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective platforms.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use (RTU) Abs
rmAb clone **MRQ-40** (prod. no. 760-4595, Ventana/Cell Marque): Protocols with optimal results were typically based on HIER using mild or standard CC1, 20-32 min incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 11 of 12 (92 %) laboratories produced produced a sufficient staining (optimal or good).

rmAb clone **SP11** (prod. no. 790-4407, Ventana): Protocols with optimal results were typically based on HIER using mild or standard CC1, 16-60 min incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 16 of 21 (76 %) laboratories produced produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stainings were:

- Less successful primary antibodies
- Too low concentration of the primary antibody
- Detection systems with a low sensitivity
- Insufficient HIER - too short efficient heating time.

In this assessment and in concordance with the three previous NordiQC assessments, virtually all laboratories were able to demonstrate SYP in the peripheral nerves in the colon, whereas staining of the neoplastic cells of the SCLC, the pancreas neuroendocrine carcinoma and in particular the intestinal carcinoid was more challenging.
This pattern was seen in 78 of the 90 insufficient results (87 %). In the remaining 13 % of the insufficient results a poor signal-to-noise ratio was seen typically characterized by an excessive background staining compromising the interpretation.
An optimal result could be obtained by the mAb clones 27G12 and Snp88 and the rmAb clones MRQ-40 and SP11. Efficient HIER in an alkaline buffer was mandatory to obtain an optimal staining result.
The proportion of optimal results was highly influenced by the IHC stainer platform. For the most commonly used Ab,, mAb clone 27G12 and applied on the Dako Autostainer system optimal results were achieved in 54 % of laboratories (mAb concentration range 1:50-200, HIER in TRS high pH 9) table 2. In comparison optimal results were only obtained in 4 % (n= 1 of 26) of laboratories using the same clone and similar protocol settings on the Ventana BenchMark platform. Several parameters could contribute to this significant difference e.g. sensitivity of detections systems used and/or impact of other reagents
The rmAb clones SP11 and MRQ-40 showed a superior performance on the Ventana BenchMark platform compared to the mAb clone 27G12. The two clones were mainly used as RTU formats and gave a proportion of optimal results of 26 % and 38 %, respectively.
The mAb clone SY38 gave insufficient results in all 32 protocols despite similar protocol settings to other Abs. In general, reduced sensitivity was seen and for unexplained reasons aberrant staining pattern was observed. In the intestinal carcinoid, coarse granular intracytoplasmic staining was seen in scattered neoplastic cells, while the majority were unstained. In all other Abs, the majority of neoplastic cells showed a distinct cytoplasmic staining reaction.

Controls
It is difficult to identify a reliable control for SYP. Normal nerves express high concentrations of SYP and cannot be used to identify a protocol with a low sensitivity. At present the best recommendation is still to use colon as control and to calibrate the protocol to give the strongest possible staining reaction of the axons of the Auerbach's and Meissner's plexus and in the endocrine cells of the mucosa. The majority of goblet cells in the mucosa must show an at least weak to moderate cytoplasmic staining reaction. No staining must be seen in the smooth muscle cells.
The reason for the staining of goblet cells in the colon mucosa is still not known. However, all Abs gave this staining pattern and as these cells only showed a weak to moderate staining reaction, the capability to identify SYP in the goblet cells is thus more reliable as critical staining quality indicator for SYP.

Effect of external quality assessment
This was the 4th NordiQC assessment of SYP. Virtually identical pass rates have been seen in the last three assessments, see table 3.

Table 3. **Proportion of sufficient results for SYP in the four NordiQC runs performed**

	Run 18 2006	Run 22 2008	Run 29 2010	Run 37 2013
Participants, n=	94	113	151	213
Sufficient results	68 %	58 %	55 %	58 %

Several parameters may contribute to the low pass rate and lack of improvement. The persistent and extensive use of the mAb clone SY38, which in the last assessments had shown a significant inferior performance have a high impact. In this assessment, mAb clone SY38 was used by 15 % of the participants and all produced an insufficient result.
The challenge to identify robust positive control with low SYP expression also complicates the validation and set-up of a high sensitivity protocols.

In this run, differences in pass rates were observed between laboratories participating for the first time and laboratories also participating in the latest assessment (run 29, 2010). Pass rates for first time participants were 43 % (32 of 74 laboratories) and 66 % (91 of 139 laboratories) of laboratories participating in both runs. Tailored recommendations for protocol improvement given to laboratories with an insufficient mark seemed to have a positive impact. 60 laboratories who had received recommendations in run 29 also submitted stainings for this run. 40 laboratories followed the recommendations, and out of these, 28 (70 %) improved to a sufficient result. 20 laboratories did not change their protocol, and out of these only 2 (10 %) improved to a sufficient result.

In conclusion, the relative low pass rate in this run was mainly caused by the increased number of new participants and persistent use of less successful Abs but also in part of the challenge to identify a robust positive control with a low expression of SYP.

Conclusion

The mAb clones **27G12** and **Snp88** and the rmAb clones **MRQ-40** and **SP11** could be used to give an optimal result for SYP. Irrespective of clone, HIER in an alkaline buffer is mandatory to give an optimal staining reaction and concentration of the primary Ab must be carefully calibrated. The performance of the Abs seems to be influenced by the stainer platform. All four Abs gave an optimal result on the Dako Autostainer platform, whereas the two rmAb clones gave a higher proportion of optimal results compared to the two mAb clones on the Ventana BenchMark platform.
Although not optimal, normal colon seems to be the most recommendable control tissue: Endocrine cells and axons of all peripheral nerves in both the muscularis propria and lamina propria must show a strong distinct granular reaction, while the majority of the epithelial goblet cells should be staining at least moderately. No staining reaction must be seen in the smooth muscle cells.

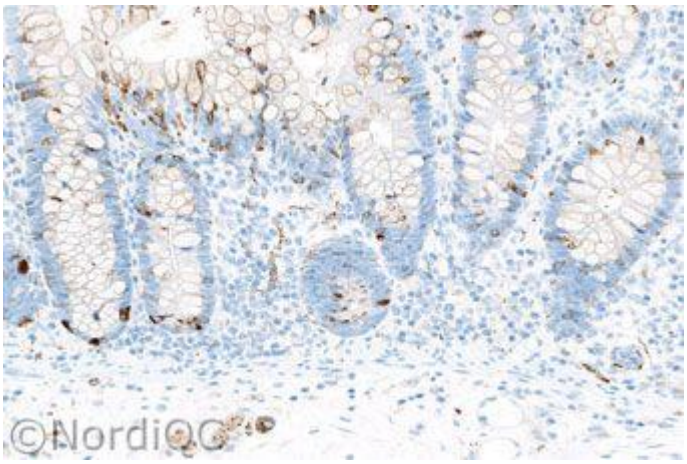

Fig. 1a
Optimal SYP staining of the colon using the rmAb clone MRQ-40, optimally calibrated and with HIER in an alkaline buffer. The peripheral nerves and the neuroendocrine cells show a strong and distinct cytoplasmic staining reaction, while the smooth muscle cells are negative. Scattered goblet cells show a weak to moderate cytoplasmic staining reaction.
Also compare with Figs. 2a - 4a – same protocol.

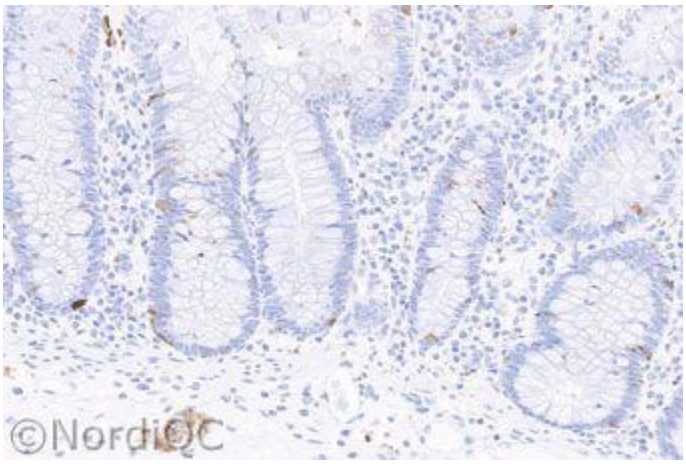

Fig. 1b
Insufficient SYP staining of the colon using the mAb clone Snp88 by protocol settings giving a too low sensitivity (too low concentration and/or less sensitive detection system) – same field as in Fig. 1a.
The peripheral nerves are distinctively demonstrated, while the proportion and intensity of the staining reaction in the neuroendocrine cells is reduced. No staining reaction is seen in the goblet cells.
Also compare with Figs. 2b & 3b – same protocol.

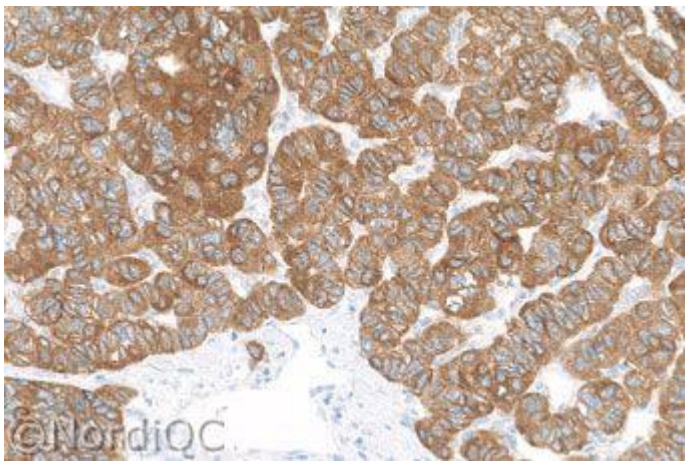

Fig. 2a
Optimal SYP staining of the pancreatic neuroendocrine carcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct staining reaction. No background staining is seen.

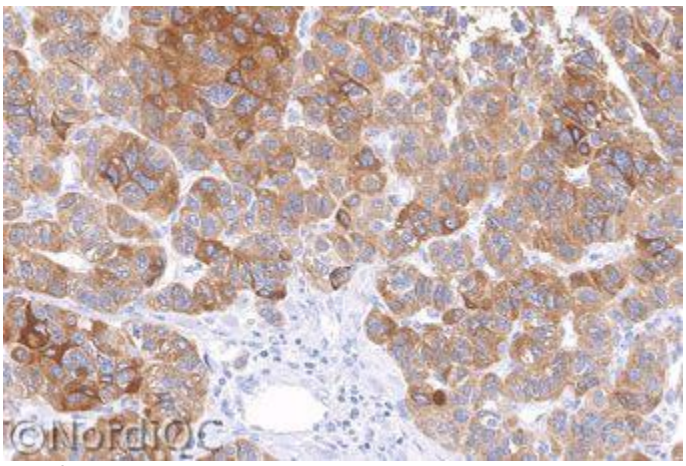

Fig. 2b
Staining for SYP of the pancreatic neuroendocrine carcinoma using same insufficient protocol as in Fig. 1b - same field as in Fig 2a. The vast majority of the neoplastic cells are demonstrated. However also compare with Fig. 3b – same protocol.

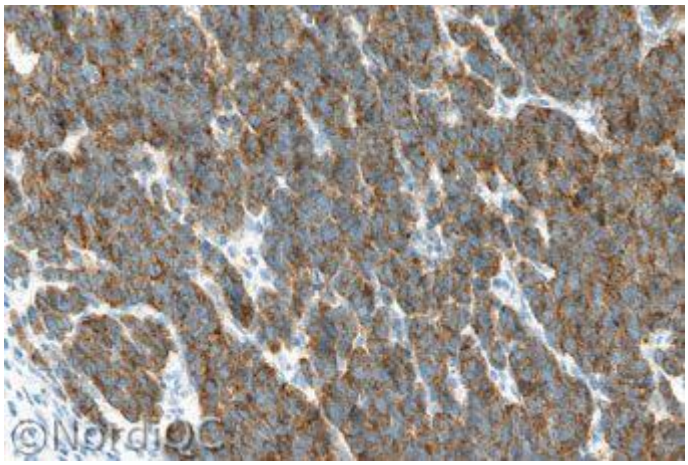

Fig. 3a
Optimal SYP staining of the SCLC using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction with a dot-like accentuation. No background staining is seen.

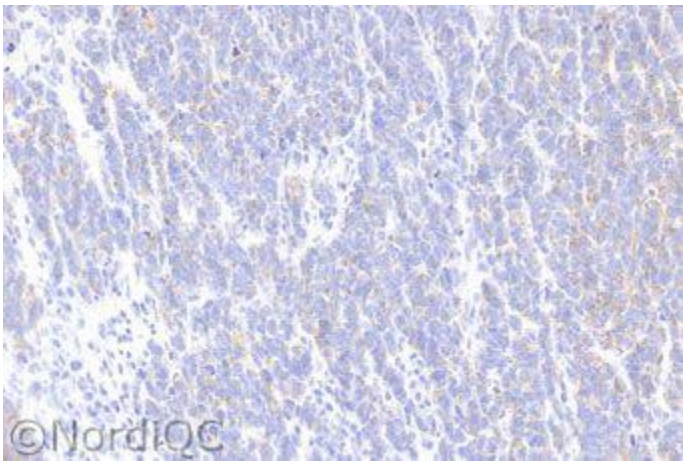

Fig. 3b
Insufficient staining SYP of the SCLC using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a.
Only scattered neoplastic cells show a weak and diffuse cytoplasmic staining reaction.

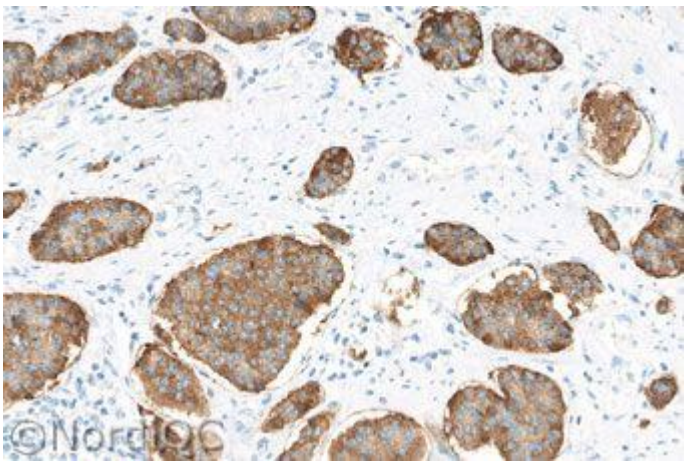

Fig. 4a
Optimal SYP staining of the intestinal carcinoid using same protocol as in Figs. 1a – 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining reaction. No background staining is seen.

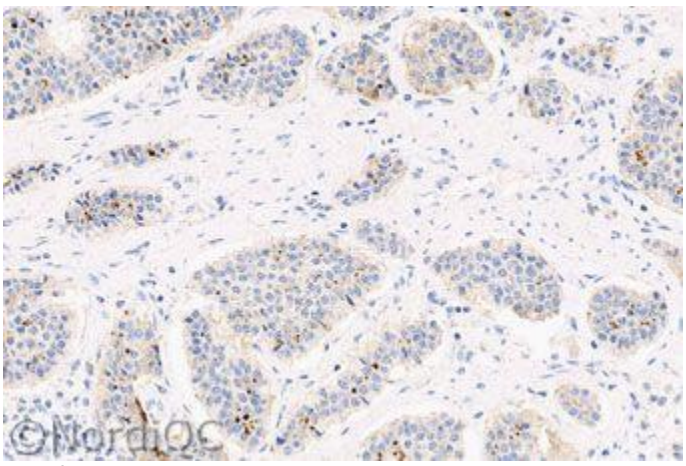

Fig. 4b
Insufficient SYP staining of the intestinal carcinoid using the mAb clone SY38. – same field as in Fig. 4a.
Only dispersed neoplastic cells show a weak and equivocal staining reaction, significantly inferior to the result expected and obtained in Fig. 4a.
In this assessment, 32 of 32 results based on this clone were assessed as insufficient due to a too weak or false negative staining.

SN/RR/LE 12-3-2013