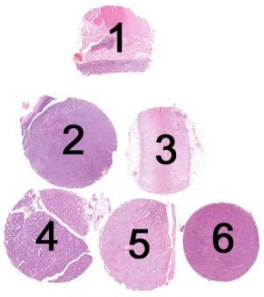
Assessment Run 29 2010

Synaptophysin (SYP)



The slide to be stained for SYP comprised:

1. Colon, 2. Small cell lung carcinoma, 3. Adrenal gland, 4. Colon adenocarcinoma, 5. Medullary thyroid carcinoma, 6. Lung carcinoid
All tissues were fixed in 10% neutral buffered formalin 24-48 h.

Criteria for assessing a SYP staining as optimal included:

- A moderate to strong, distinct cytoplasmic reaction of the normal neuroendocrine cells. A weak to moderate staining of the goblet cells was accepted.
- At least a moderate, distinct granular cytoplasmic reaction of the normal ganglion cells and the axons of the nerve plexus in the colon.
- A moderate to strong, distinct cytoplasmic, dot-like reaction in virtually all the cortical epithelial cells of the adrenal gland.
- At least a moderate, distinct cytoplasmic, dot-like reaction in the majority of the neoplastic cells of the small cell lung carcinoma and the medullary thyroid carcinoma.
- Scattered positive cells in the colon adenocarcinoma – while the large majority of tumour cells should be negative.

151 laboratories participated in this assessment. 55 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for SYP, run 29**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb[3)] clone **27G12**	60 1	Novocastra / Leica Monosan	19	28	8	6	77 %	78 %
mAb clone **Snp88**	18	BioGenex	4	8	3	3	67 %	83 %
mAb clone **SY38**	8	Dako	0	0	2	6	0 %	0 %
rmAb clone **SP11**	8 2 1 1	NeoMarkers Spring Bioscience Master Diagnostica Unknown	2	6	1	3	67 %	100 %
rmAb clone **Z66**	1	Zymed	0	1	0	0	-	-
pAb **A0010**	12	Dako	0	2	4	6	17 %	-
pAb **CMC111**	2	Cell Marque	0	0	2	0	-	-
pAb **NCL-Synapp**	2	Novocastra / Leica	0	0	1	1	-	-
pAb **RB-1461**	1	NeoMarkers	0	1	0	0	-	-
pAb **SIGNET-3261-1000**	1	Signet Lab	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **SY38, IR776**	10	Dako	0	1	0	9	10 %	-
mAb clone **SY38, N1566**	1	Dako	0	0	0	1	-	-
mAb clone **27G12, RTU-Synap-299 /PA0299**	3	Novocastra / Leica	0	2	1	0	-	-
mAb clone **27G12, PM371**	1	BioCare	0	1	0	0	-	-
rmAb clone **SP11, 760-4407**	6	Ventana	1	2	2	1	50 %	100 %

rmAb clone **MRQ-40, 336R-97**	1	Cell Marque	1	0	0	0	-	-
pAb **760-2668**	9	Ventana / Cell Marque	0	2	7	0	22 %	-
pAb **CMA110**	1	Cell Marque	1	0	0	0	-	-
pAb **RB-1461-R7**	1	NeoMarkers	0	0	0	1	-	-
Total	151		28	54	31	38	-	-
Proportion			19 %	36 %	20 %	25 %	55 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, rmAb: rabbit monoclonal antibody, pAb: polyclonal antibody.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **27G12**: The protocols giving an optimal result were all based on HIER with either Tris-EDTA/EGTA pH 9 (4/13)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (8/16), Cell Conditioning 1 (BenchMark, Ventana) (5/17), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/7) or Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/2) as the 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 39 out of 50 laboratories (77 %) produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffe)

mAb clone **Snp88**: The protocols giving an optimal result were based on HIER with Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (3/7) or Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:100–1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 6 laboratories (83 %) produced a sufficient staining (optimal or good).

rmAb clone **SP11**: The protocols giving an optimal result was based on HIER with Cell Conditioning 1 (BenchMark, Ventana) (1/4) or Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (1/1) as the retrieval buffer. The mAb was diluted in the range of 1:40 -1:1:200. Using these protocol settings all of 6 (100 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
rmAb clone **SP11** (prod.no. 760-4407, Ventana): The protocol giving an optimal result was based on HIER using Cell Conditioning 1 extended (BenchMark, Ventana), an incubation time of 44 min in the primary Ab and UltraView (760-500) + amplification as the detection system.

rmAb clone **MRQ-40** (prod.no. 336R-97, Cell Marque): The protocol giving an optimal result was based on HIER with Cell Conditioning 1 mild (BenchMark, Ventana), an incubation time of 16 min in the primary Ab and Ventana UltraView as the detection system.

pAb prod. no. **CMA110**, Cell Marque: The protocol giving an optimal result was based on HIER with Bond Epitope Retrieval Solution 1 (Bond, Leica), an incubation time of 16 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system.

The most frequent causes of insufficient staining were:

- Less successful primary antibodies
- Too low concentration of the primary antibody
- Insufficient HIER - too short efficient heating time.

In this assessment the prevalent feature of an insufficient staining was a generally too weak or false negative staining reaction of the cells expected to stain. This was seen in 47/69 of the insufficient results (68 %) and was mainly caused by a too low concentration of the primary Ab and/or insufficient HIER, but also related to the Ab applied: The mAb clone SY38 gave an insufficient staining in 16 out of 17 protocols. Virtually all laboratories could demonstrate SYP in the neuroendocrine cells of the colon and in the lung carcinoid, whereas the demonstration of SYP in the small cell lung carcinoma and the medullary thyroid carcinoma was more challenging and required a carefully calibrated protocol. In 22/69 of the cases (32 %) a false positive staining reaction was observed, typically seen with the 2 pAbs A0010 (Dako) and 760-2668 (Ventana), characterized by a moderate granular cytoplasmic reaction in the epithelial cells of the colon and the colon adenocarcinoma.
The choice of Ab thus had a high impact on the pass rate. While the proportion of sufficient stains with clone

27G12 was 76 %, it was 0 % with clone SY38, despite similar protocol settings were applied. In Table 2, the overall pass rates for the five most used clones in the last three SYP assessments are summarized.

Table 2. **Performance of the five most commonly used Abs in three runs for SYP**

	Run 18 2006		Run 22 2008		Run 29 2010		Total	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone 27G12	14	12	22	19	65	50	101	81 (80 %)
mAb clone Snp88	14	14	16	16	18	12	48	42 (88 %)
mAb clone SY38	11	3	11	0	19	1	41	4 (10 %)
pAb A0010	42	23	39	16	12	2	93	41 (44 %)
pAb 760-2668	6	4	10	4	9	2	25	10 (40 %)

The mAb clones 27G12 and Snp88 have shown to be superior to the three other most commonly used Abs for SYP. However, clone Snp88 (BioGenex) is still only produced as an ascites format which may give a MAG reaction mimicking the true SYP reaction in endocrine tumours (as described in SYP run 22). In this run a few stains based on the clone 27G12 gave an aberrant cytoplasmic granular reaction in the colon adenocarcinoma. No single specific explanation (e.g., lot-to-lot variation, Ab titre, detection system) could be identified as the cause, but the combination of efficient HIER and usage of a 3-step labelled detection system as EnVision Flex+ seemed to cause the aberrant and unexpected reaction.

In this assessment new Abs were used, e.g., rmAb clone SP11 and rmAb clone MRQ-40. Both could give an optimal staining. Future assessments may provide more data about the robustness of these Abs.

It is difficult to identify a reliable positive control for SYP. Normal nerves express a high concentration of SYP and can not be used to identify a protocol with a low sensitivity. At present the best recommendation is still to use appendix/colon as control and to calibrate the protocol to give an as strong as possible reaction in the axons of the Auerbach's and Meissner's plexus and also to see a distinct staining in the endocrine cells.

This was the 4th NordiQC assessment of SYP. A relatively constant, but too low proportion of sufficient results have been seen in the latest two runs (table 3), which in part can be explained by the new labs, many of which still use clone SY38 or one of the polyclonals.

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

	Run 5 2002	Run 18 2006	Run 22 2008	Run 29 2010
Participants, n=	39	94	112	151
Sufficient results	74 %	68 %	58 %	55 %

Conclusion

In this assessment, the mAb clones 27G12 and Snp88 and the rmAb clones SP11 and MRQ-40 could be used to give an optimal staining for SYP. HIER is mandatory to give an optimal staining and the concentration of the primary Ab should be carefully calibrated. Normal appendix/colon seems to be the most recommendable control tissue: The endocrine cells and the axons of all the peripheral nerves in both the muscularis propria and lamina propria must show a strong distinct granular reaction, scattered epithelial goblet cells at least a moderate staining, while the smooth muscle cells must be negative.

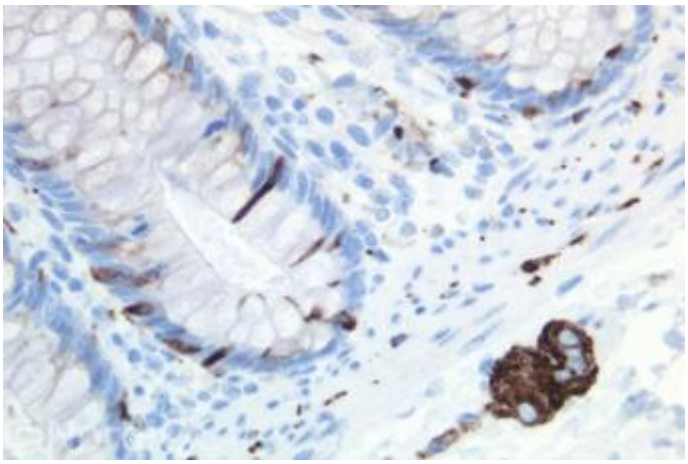


Fig. 1a
Optimal SYP staining of the appendix using the mAb clone 27G12. The peripheral nerves and the neuroendocrine cells show a strong and distinct cytoplasmic staining. The smooth muscle cells are negative. Scattered goblet cells show a weak cytoplasmic staining. Also compare with Figs. 2a & 3a – same protocol.

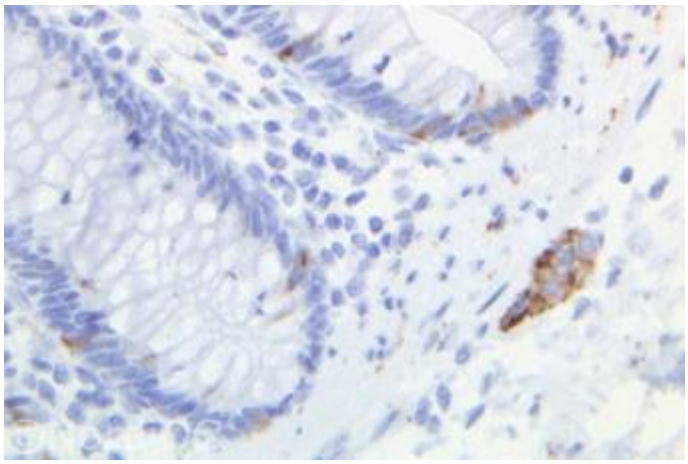


Fig. 1b
Insufficient SYP staining (borderline) of the appendix based on the mAb clone 27G12 used too diluted – same field as in Fig. 1a. The peripheral nerves are distinctively demonstrated, while a reduced proportion of the neuroendocrine cells show a weak and diffuse staining. Also compare with Figs. 2b & 3b – same protocol.

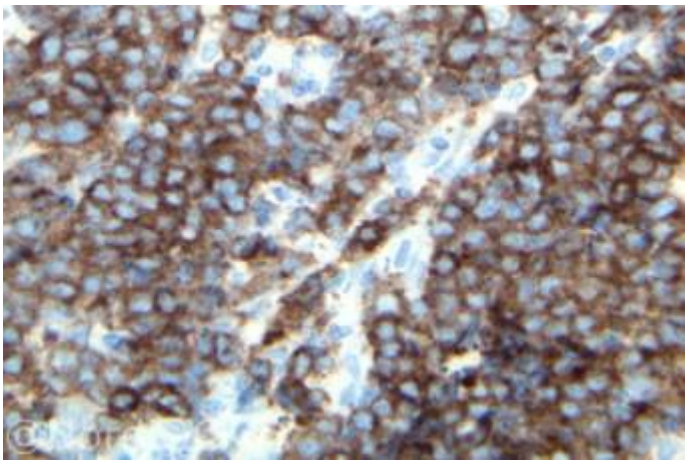


Fig. 2a
Optimal SYP staining of the SCLC using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct staining.

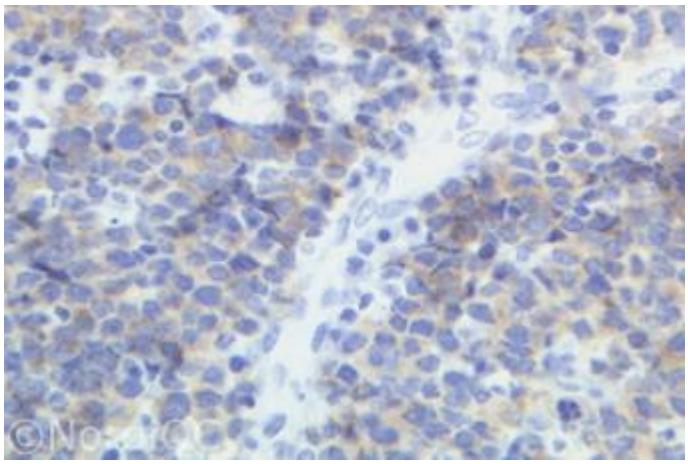


Fig. 2b
Insufficient SYP staining of the SCLC using same protocol as in Fig. 1b - same field as in Fig 2a. The neoplastic cells only show a weak or equivocal staining. Also compare with Fig. 3b – same protocol.

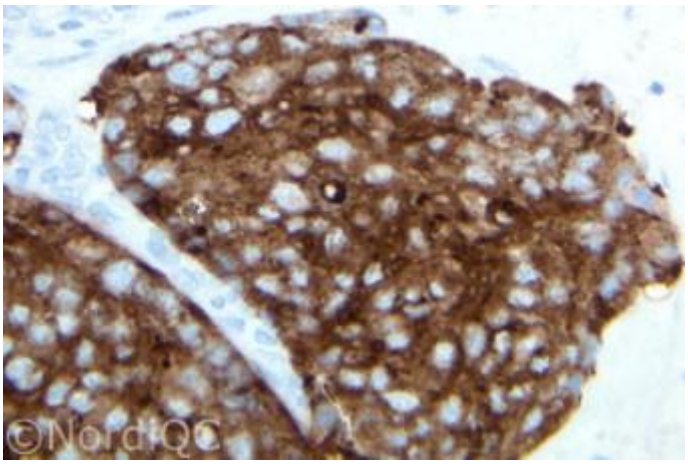


Fig. 3a
Optimal SYP staining of the medullary thyroid carcinoma using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct staining.

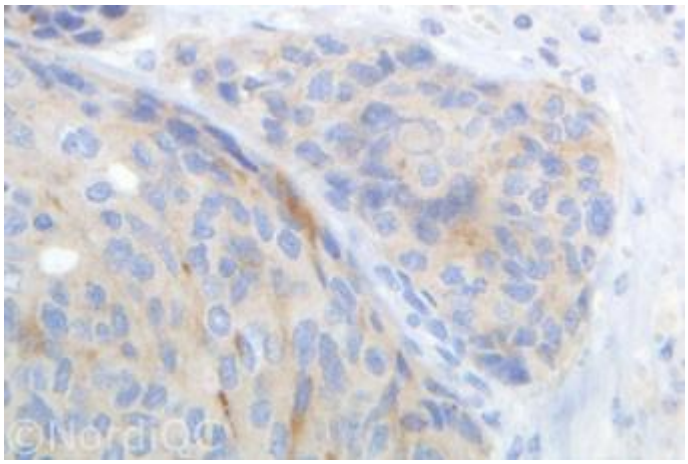


Fig. 3b
Insufficient SYP staining of the medullary thyroid carcinoma using same protocol as in Figs. 1b & 2b.
Only scattered neoplastic cells show a weak or equivocal, diffuse cytoplasmic staining.

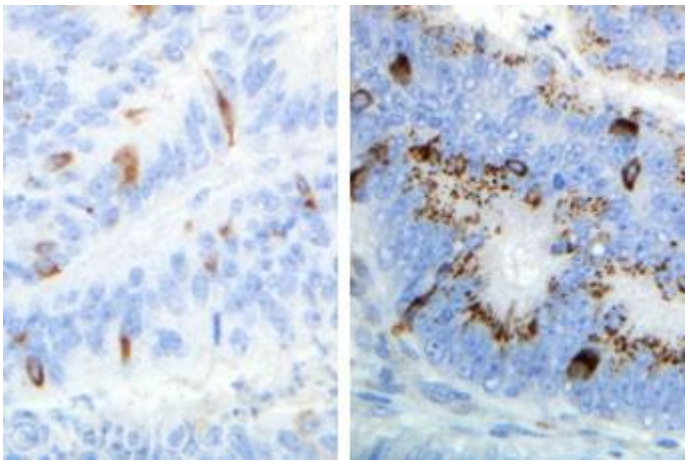


Fig. 4a
SYP staining of the colon adenocarcinoma using the mAb clone 27G12 visualized with a 2-step and a 3-step polymer based detection system respectively – same lot L129939, same concentration 1:50, and same HIER conditions in TE pH 9,
Left: EnVision Flex K8000, Dako 30 min.
The neuroendocrine cells show a distinct sytoplasmic staining, while the neoplastic cells are unstained.
Right: EnVision Flex+ K8002, Dako 15 + 30 min.
The majority of the neoplastic cells show a moderate cytoplasmic staining – entrapped normal neuroendocrine cells show a strong staining.
The aberrant cytoplasmic staining was typically seen when the clone 27G12 was applied relatively concentrated, with efficient HIER and using a 3-step polymer based system.

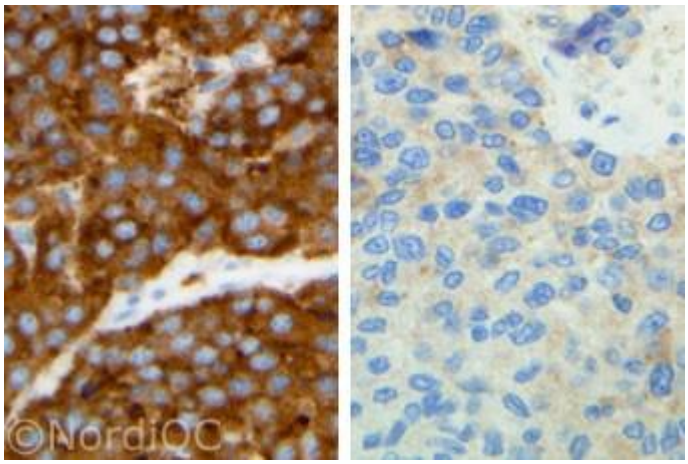


Fig. 4b
Insufficient SYP staining for SYP using the mAb clone SY38. 16/17 protocols based on this clone was assessed as insufficient due to a too low sensitivity in the SCLC and medullary thyroid carcinoma.
Left: Carcinoid: Virtually all the neoplastic cells show a strong cytoplasmic staining. This tumour has a high expression of SYP and was strongly positive with all of protocols based on the clone SY38.
Right: Medullary thyroid carcinoma: Only scattered neoplastic cells show a weak and diffuse cytoplasmic staining. This tumour was virtually negative in 16/17 protocols based on the mAb clone SY38.

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