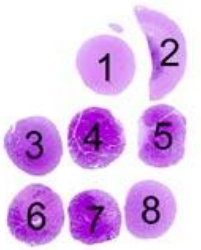
Assessment Run 22 2008

Synaptophysin (SYP)



The slide to be stained for Synaptophysin (SYP) comprised:
1. Brain, 2. Appendix, 3. Colon adenocarcinoma, 4. Pancreas blood type A, 5. Pancreas blood type 0, 6-7. Endocrine lung carcinomas, 8. Medullary thyroid carcinoma.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a SYP staining as optimal included:

- A strong and distinct cytoplasmic reaction of the normal neuroendocrine cells in the appendiceal mucosa and in the islet cell in the pancreas (a weak background reaction around the islets was accepted due to diffusion of the protein in the two pancreas specimens).
- At least a moderate, distinct granular cytoplasmic reaction of the normal ganglion cells and axons in the appendiceal Aurbach's plexus and in the brain.
- At least a moderate, distinct cytoplasmic reaction in the majority of the neoplastic cells of the two endocrine lungcarcinomas.
- A strong and distinct cytoplasmic reaction of the medullary thyroid carcinoma.
- Scattered positive cells in the colon adenocarcinoma – while the large majority of tumour cells should be negative.

113 laboratories submitted stains. One laboratory used an inappropriate antibody. At the assessment of 112 laboratories 20 achieved optimal marks (18 %), 45 good (40 %), 37 borderline (33 %) and 10 poor marks (9 %).

The following Abs were used:
mAb clone **27G12** (Novocastra, n=22)
mAb clone **Snp88** (BioGenex, n=16)
mAb clone **SY38** (Dako, n=10; BioGenex, n=1)
rmAb clone **SP11** (NeoMarkers, n=6)
pAb **A0010** (Dako, n=39)
pAb **N1566** (Dako, n=3)
pAb **760-2668** (Ventana, n=10)
pAb **18-0130** (Zymed, n=3)
pAb **NCL-SYNAPp** (Novocastra, n=1)
pAb **RB-1461** (NeoMarkers, n=1)

Optimal staining for SYP in this assessment was obtained with the mAb clone **27G12** (11 out of 22), the mAb clone **Snp88** (6 out of 16), the pAb **A0010** (1 out of 39) and the pAb **18-130** (2 out of 3).

27G12: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (7/11)*, Citrate pH 6 (2/2) or Target Retrieval Solution pH 9 (FLEX TRS high pH, Dako) (1/1). The mAb was typically diluted in the range of 1:30 – 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 out of 14 (100 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer).

Snp88: The protocols giving an optimal result were all based on HIER using Tris-EDTA/EGTA pH 9 (4/10) or Bond Epitope Retrieval Solution 2 (Bond, Leica Microsystems; 2/2). The mAb was diluted in the range of 1:100 – 1:3,000 depending on the total sensitivity of the protocol employed. Using these protocol settings 11 out of 11 (100 %) laboratories produced a sufficient staining.

18-0130: The protocols giving an optimal result were based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 (1/1) or no retrieval (1/1). The mAb was diluted in the range of 1:50 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 out of 2 (100 %) laboratories produced a sufficient staining (optimal or good).

A0010: The protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (1/28). The pAb was used in the dilution 1:100. Using these protocol settings 5 out of 7 (71 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low concentration of the primary antibody

The prevalent features of the insufficient results were 1) A generally too weak or false negative reaction (66 %), 2) An excessive background reaction (11 %), or 3) A combination of a weak reaction and excessive background reaction giving a poor signal-to-noise ratio (23 %).

One of the central parameters for a sufficient SYP demonstration was the choice of the primary antibody.
In this assessment the mAb clone SY38 gave an insufficient reaction in 11 out of 11 protocols. The laboratories using SY38 typically obtained an acceptable reaction in the normal endocrine cells and peripheral nerves in the appendix, but a false negative reaction in large proportions of the neoplastic cells in the endocrine lung carcinomas, sometimes also in the medullary thyroid carcinoma.
The false positive reactions were typically observed when the 2 pAbs A0010 (Dako) and 760-2668 (Ventana) were used. The pAb A0010 mainly gave a general background reaction e.g a cytoplasmic cross reaction in the appendiceal enterocytes and of unexplained reasons also a strong nuclear reaction in both the pancreas and the colon adenocarcinoma.
The pAb 760-2688 typically gave a non-specific dot-like cytoplasmic reaction in several cell types as the appendiceal enterocytes, the acinar pancreatic cells, and the colon adenocarcinoma. Laboratories trying to reduce this non-specific reaction by diluting the antibody obtained too weak reactions of the neuroendocrine tumours.

In the multitissue block used for the assessment in this run two pancreas specimens from patients with blood group 0 and blood group A, respectively. In stains with mAb clone Snp88, a distinct dot-like cytoplasmic reaction of the acinar cells in the pancreas from the patient with blood group A was seen. This pattern, which has been described in the literature as "Mouse Antibody Golgi" (MAG), is undesired and the interpretation using such Abs has to be taken with care, as the pattern highly mimics the SYP reaction in endocrine tumours. NordiQC will contact the producer of the ascites mAb suggesting a change to a supernatant Ab to reduce this source of error.

The overall pass rates for the most used Abs for SYP in the latest two assessments are shown in the table:

	Run 18 2006		Run 22 2008		Total	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb clone 27G12	14	12	22	19	36	31 (86%)
mAb clone Snp88	14	14	16	16	30	30 (100%)
mAb clone SY38	11	3	11	0	22	3 (14%)
pAb A0010	42	23	39	16	81	39 (48%)
pAb 760-2668	6	4	10	4	16	8 (50%)

These data clearly indicates the most robust markers for SYP are the clones 27G12 and Snp88.

From the assessment it can be difficult to identify a robust and reliable control for SYP. Appendix was in run 18 found to a recommendable control, in which the peripheral nerves should display a moderate to strong reaction. However, it appears that mAb clone SY38 can give a moderate to strong staining of the peripheral nerves and still leave the endocrine carcinomas too weak or even false negative.

At present the best recommendation is still to use appendix as control but defer from using mAb SY38, and to calibrate the primary Ab to give a strong reaction in the peripheral nerves in the Auerbach's and Meissner's plexus as well as the axons in lamina propria beneath the epithelial cells. Muscle cells must remain negative, whereas scattered epithelial cells may be positive.

Compared to the assessment of SYP in run 18 2006, an decrease in the proportion of sufficient results from 69 % to 58 % was seen. This decrease may be due to many new participants and the continuous use of less successful antibodies.
25 laboratories, which obtained an insufficient result in run 18, submitted a new SYP stain in run 22. 14 of these followed the recommendation given and 9 improved their marks to good or optimal (64 %). 11 laboratories did

not follow the recommendation, and only 1 of these (9 %) obtained a sufficient staining in run 22.

Conclusion
In this assessment, the mAb clones 27G12 and Snp88 were the most robust Abs for the demonstration of SYP. However, mAb clone Snp88 used in an ascites format can give an undesired cross reactivity in sensitive protocols.
The concentration of the primary Ab should be carefully calibrated. Normal appendix seems to be the most recommendable control tissue: the axons of all the peripheral nerves in both the muscularis propria and lamina propria must show a strong distinct granular reaction, while the smooth muscle cells being negative.

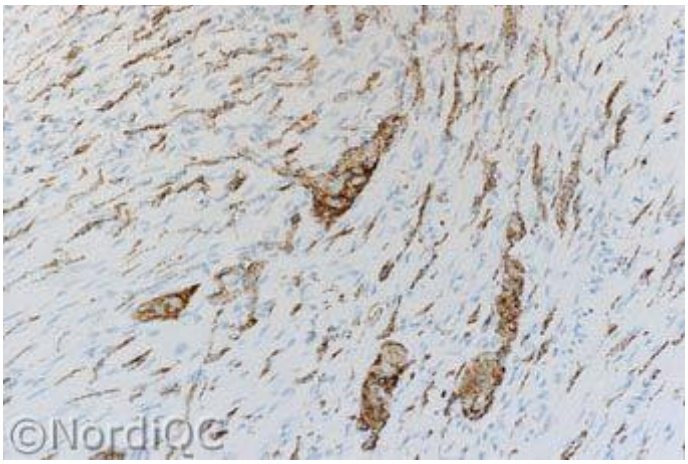


Fig. 1a
Optimal staining for SYP of the appendix using the mAb clone 27G12. Both the axons and perikaryonic compartment of the peripheral nerves in tunica muscularis show a distinct cytoplasmic reaction, while the smooth muscle cells are negative.

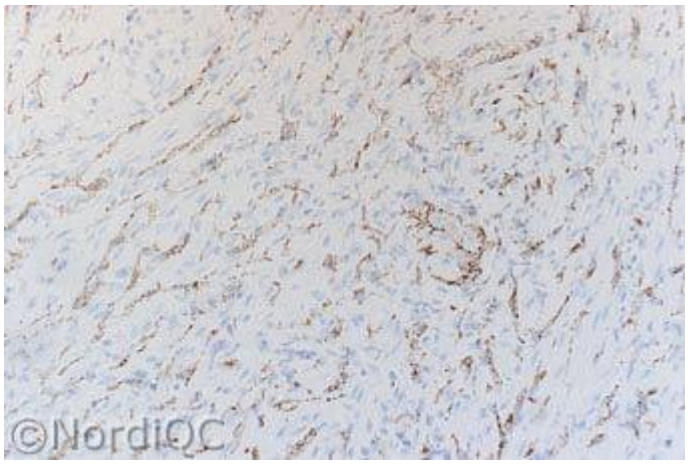


Fig. 1b
SYP staining – same field as in Fig. 1a – using an insufficient protocol based on the mAb clone SY38. The axons of the peripheral nerves are demonstrated, but showing a weaker reaction compared to Fig 1a. Also compare with Fig. 2b – same protocol.

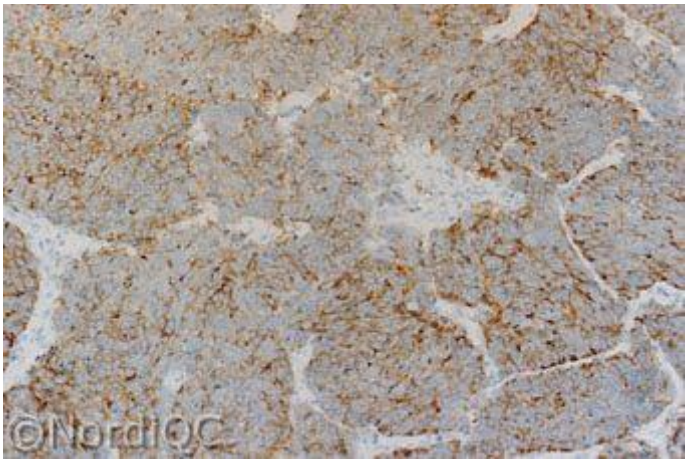


Fig. 2a
Optimal SYP staining of the endocrine lung carcinoma no. 6 using same protocol as in Fig. 1a. The majority of the neoplastic cells show a distinct staining and a scattered dot-like reaction.

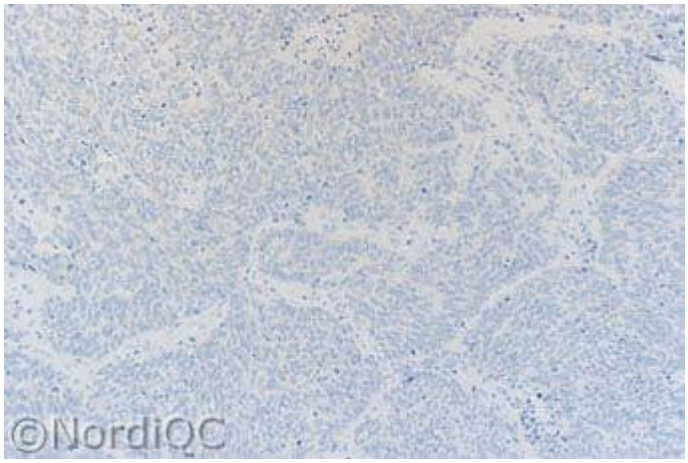


Fig. 2b
Insufficient SYP staining of the endocrine lung carcinoma no. 6 using same protocol as in Fig. 1b. The neoplastic cells are virtually negative.

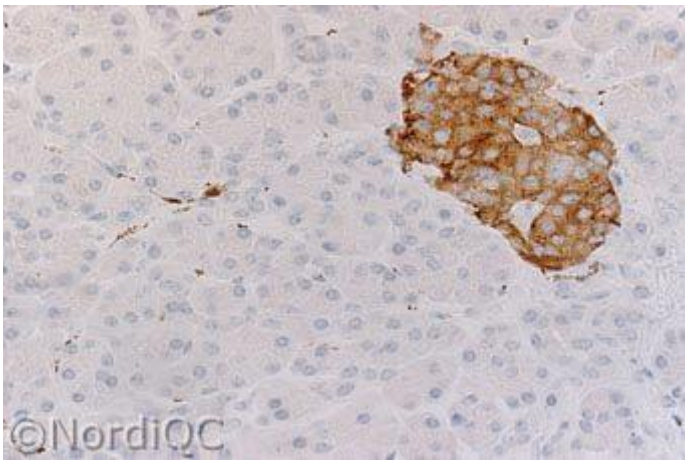


Fig. 3a
SYP staining of the pancreas blood type 0 using the ascites harvested mAb clone Snp88. The endocrine cells in the islets show a distinct reaction, while the acinar cells are negative. Compare with Fig 3b – blood type A, same protocol.

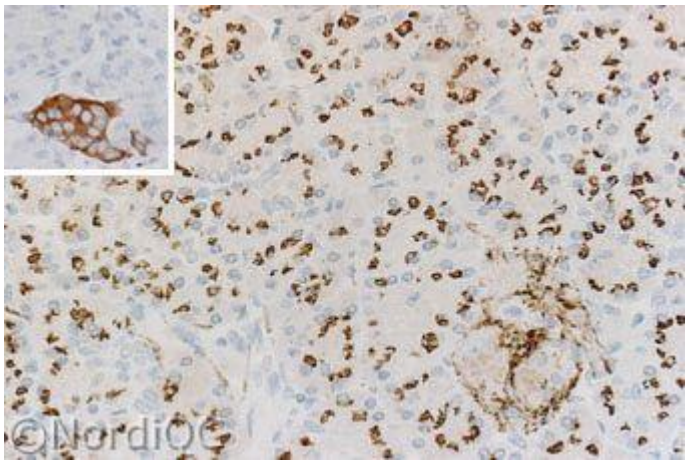


Fig. 3b
SYP staining of the pancreas blood type A using the ascites harvested mAb clone Snp88. The acinar cells show a distinct dot-like cytoplasmic reaction as the Ab cross reacts with a golgi associated glycoprotein (MAG).
Insert same pancreas stained with the mAb supernatant clone 27G12 – no reaction is observed.

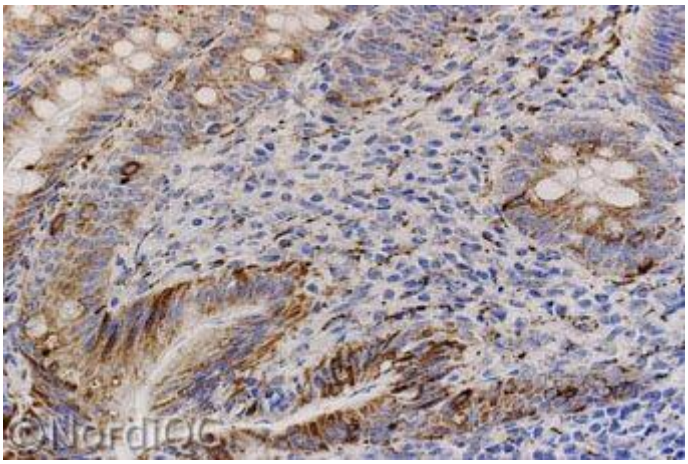


Fig. 4a
Insufficient staining for SYP of the appendix using the pAb A0010. The nerves are demonstrated, but excessive false positive reaction of enterocytes compromises the interpretation. This reaction pattern and the pattern demonstrated in Fig 4b was frequently observed with the pAb.

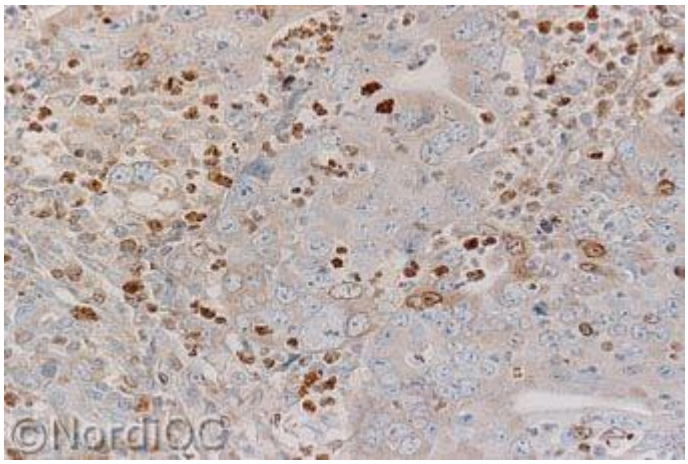


Fig. 4b
Insufficient staining for SYP of the colon adenocarcinoma using the pAb A0010. An aberrant nuclear reaction is seen in both inflammatory cells and the neoplastic cells and the latter also show a diffuse cytoplasmic staining.

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