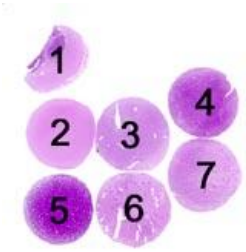
## Assessment Run 18 2006

# Synaptophysin (SYP)




The slide to be stained for synaptophysin (SYP) comprised:
1. Appendix, 2. Brain, 3. Neuroendocrine carcinoma, 4. Small cell lung carcinoma (SCLC), 5. Merkel cell carcinoma, 6. Colon adenocarcinoma, 7. Thyroid medullary carcinoma. All specimens were fixed in 10 % NBF.

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 colon adenocarcinoma.
- A moderate to strong distinct granular cytoplasmic reaction of the normal axons of the nerves in the appendiceal lamina propria and muscularis propria, and in the brain.
- A distinct cytoplasmic reaction in the majority of the neoplastic cells of the SCLC, the Merkel cell carcinoma, the large cell neuroendocrine carcinoma and the thyroid medullary carcinoma.
- No staining of the majority of the neoplastic cells in the colon adenocarcinoma.

94 laboratories participated in the assessment. 24 achieved optimal marks (25 %), 40 good (43 %), 22 borderline (23 %) and 8 (8 %) poor marks.

The following Abs were used:
mAb clone **27G12** (Novocastra n=14)
mAb clone **Snp88** (BioGenex n=14)
mAb clone **SY38** (Dako, n=10; Cymbus, n=1)
rmAb clone **SP11** (NeoMarkers, n=3)
pAb **18-0130** (Zymed, n=2)
pAb **250-2735** (Ventana, n=1)
pAb **760-2668** (Ventana, n=5)
pAb **A0010** (Dako, n=39)
pAb **CMC11** (Cell Marque, n=1)
pAb **N1566** (Dako, n=2)
pAb **RB-1461** (NeoMarkers, n=1)
pAb **RB-9044** (NeoMarkers, n=1)

Optimal staining for SYP in this assessment was obtained with the mAb clones **27G12** and **Snp88**, the rmAb **SP11**, and the pAbs **A0010** and **RB-9044**. All optimal results were based on HIER irrespective of the Ab applied.

**27G12**: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) in either Tris-EDTA/EGTA pH 9 or Cell Conditioning 1 (CC1 Ventana). The Ab was typically diluted in the range of 1:50 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 12 out of 14 laboratories (86 %) produced a sufficient staining (optimal or good), of which 10 were optimal (71 %).

**Snp88**: the protocols giving an optimal result was based on HIER in either Tris-EDTA/EGTA pH 9, Cell Conditioning 1 (CC1 Ventana), Bond Epitope Retrieval Solution 2 (Vision BioSystems) or Citrate pH 6. The mAb was was typically diluted in the range of 1:100 – 1:4,000 depending on the total sensitivity of the protocol employed. Using similar protocol settings 14 out of 14 laboratories (100 %) produced a sufficient staining (optimal or good). 4 of these were optimal (29 %).

Clone Snp88 occasionally gave a weak nuclear staining. This did not interfere with the interpretation and consequently did not affect the assessment marks.

**SP11**: the protocol giving an optimal result was based on HIER in Citrate pH 6, the Ab diluted 1:30. Using a similar protocol setting another laboratorium obtained good marks.

**A0010**: the protocols giving an optimal result were based on HIER in either Tris-EDTA/EGTA pH 9 or Target Retrieval Solution pH 6.1 (TRS, Dako, S1699). The pAb was was typically diluted in the range of 1:50 – 1:200 depending on the total sensitivity of the protocol employed. With these protocol settings 22 out of 35 laboratories (63 %) produced a sufficient staining, of which 8 were optimal (23 %).

**RB-9044**: the protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 and an Ab

dilution of 1:300.

The most frequent causes of an insufficient staining were:
- Less successful primary antibody
- Omission of heat induced epitope retrieval (HIER)
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody

Almost all laboratories were able to demonstrate SYP in the normal appendiceal neuroendocrine cells and the large cell lung neuroendocrine carcinoma, whereas the prevalent feature of the insufficient staining was a too weak or false negative staining of the SCLC, the Merkel cell carcinoma and the medullary thyroid carcinoma. A too weak or false negative staining was seen in 71 % of the insufficient results (20 out of 28), while a too strong (false positive) staining was observed in 21 % (6 out of 28). A combination of false negative and false positive reaction was noticed in 7 % (2 out of 28), typically due to a combination of insufficient epitope retrieval and too high concentration of Ab.

The peripheral nerves in the appendiceal muscularis propria appeared to be a reliable and valid quality indicator for the immunohistochemical demonstration of SYP, as the sufficient results all showed a strong distinct reaction of SYP in the axons while the smooth muscle cells were negative. If the axons only showed a weak reaction or were negative, more of the tumors especially the SCLC and the medullary thyroid carcinoma became partly or entirely false negative. If the smooth muscle cells were positive, a general too high background reaction was observed throughout the specimens. The normal neuroendocrine cells cannot be recommended as a positive control as these cells harbour a high SYP expression.

SYP was also assessed in run 5 2002 (pilot run only).

**Conclusion**

The mAb clones 27G12, Snp88, SP11, and the pAbs A0010 and RB-9044 seem to be useful Abs for the demonstration of SYP. HIER is mandatory to obtain an optimal result. Focusing on the markers used by > 10 laboratories, the mAb clones 27G12 and Snp88 resulted in a significant higher ratio of sufficient stains (93 %) than mAb clone SY38 (27 % good but no optimal).

The concentration of the primary Ab should be carefully calibrated. Normal appendix seems to be an appropriate control tissue: the axons of the nerves in the muscularis propria must show a strong distinct granular reaction while the surrounding muscle cells being negative.

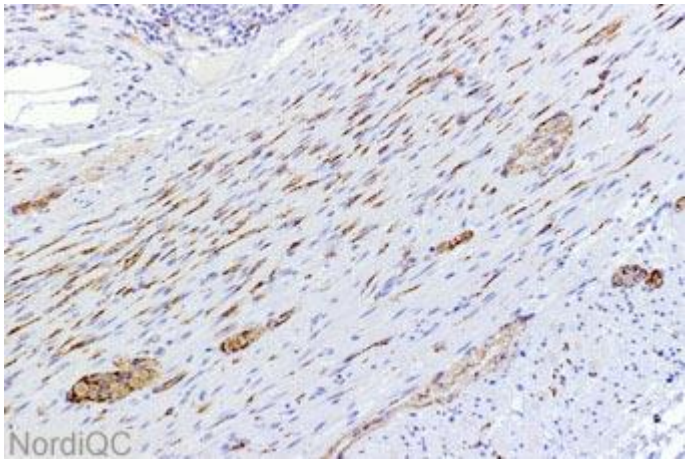



Fig. 1a
Optimal staining for SYP of the appendix using the mAb clone 27G12. The axons in tunica muscularis show a distinct cytoplasmic reaction, while the smooth muscle cells are negative.

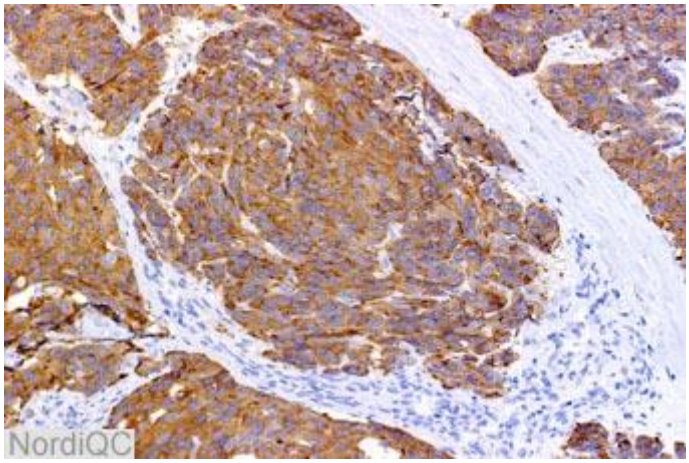



Fig. 1b
Optimal staining for SYP of the medullary thyroid carcinoma. Virtually all the neoplastic cells show a distinct cytoplasmic reaction. Same protocol as in Fig 1a.

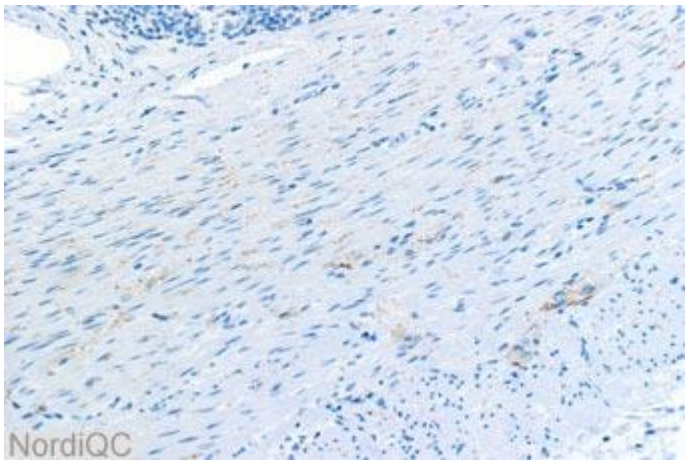



Fig. 2a
Insufficient staining for SYP of the appendix, same field as in Fig. 1a. The axons are only weakly demonstrated.

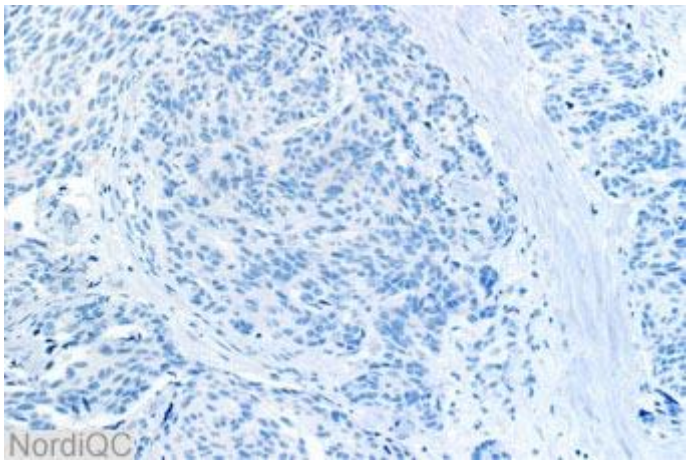



Fig. 2b
Insufficient staining for SYP of the medullary thyroid carcinoma, same field as in Fig 1b. All the neoplastic cells are false negative. Same protocol as in Fig 2a.

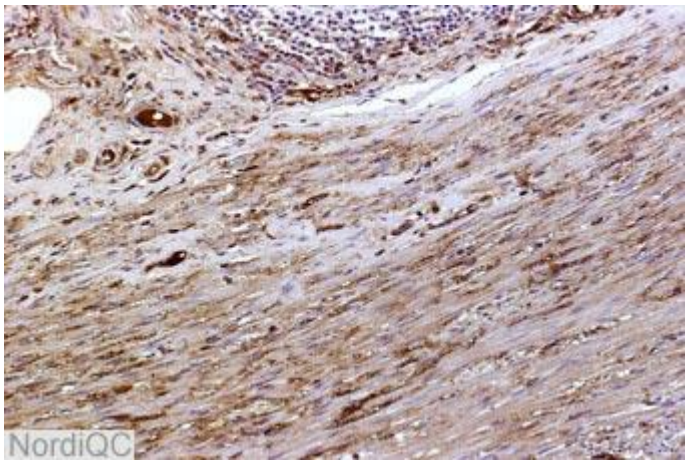



Fig. 3a
Insufficient staining for SYP of the appendix using pAb A0010, same field as in Fig. 1a. The axons are demonstrated, but the false positive reaction of the smooth muscle cells, due to a too high concentration of the primary combined with omission of HIER.

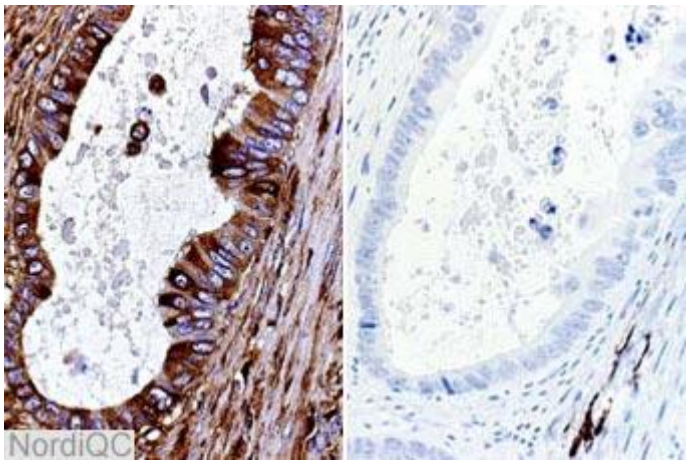



Fig. 3b
Left: Insufficient staining for SYP of the colon carcinoma using same protocol as in Fig. 3a. The high concentration of the primary Ab induces a false positive reaction of both the neoplastic cells and the stromal cells.
Right: Optimal staining for SYP of the colon carcinoma (same field as the figure to the left). The neoplastic cells are negative and only the peripheral nerves are positive.

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