Assessment Run 66 2022

Synaptophysin (SYP)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for SYP, typically used in the diagnostic work-up of neuroendocrine tumors. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for SYP (see below).

Material
The slide to be stained for SYP comprised:

1. Duodenum, 2. Neuroendocrine carcinoma, NOS, 3. Small cell lung carcinoma (SCLC), 4. Colon adenocarcinoma,

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 the ganglion cells and axons of the nerve plexus in the duodenum.
- An as strong as possible staining reaction of the Paneth and goblet cells in the duodenum mucosa[1].
- A moderate to strong, distinct, cytoplasmic staining reaction of the peripheral nerves situated in lamina propria mucosa of the duodenum.
- An at least weak to moderate, distinct, cytoplasmic staining reaction of virtually all neoplastic cells of the SCLC.
- A moderate to strong, distinct cytoplasmic staining reaction of virtually all neoplastic cells of the neuroendocrine tumour, NOS.
- No staining of neoplastic cells in the colon adenocarcinoma.

[1] Following the recommendations given by the International Ad Hoc Expert Committee (Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18.) strictly, the majority of goblet cells should display a weak to moderate staining intensity.

Participation

Number of laboratories registered for SYP, run 66	414
Number of laboratories returning slides	390 (94%)

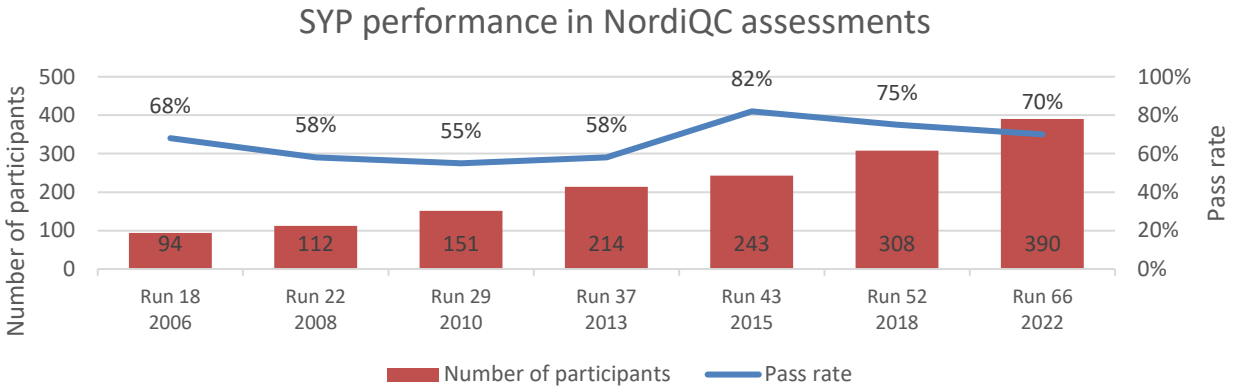
Results
At the date of assessment, 94% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

390 laboratories participated in this assessment and 70% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Inefficient HIER – too short time or use or acidic buffer.
- Too low concentration of the primary antibody or too short incubation time.
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters.

Performance history
This was the seventh NordiQC assessment of SYP. A reduced pass rate was observed compared to the two previous runs (see Graph 1), which primarily was due to extended use of Ready-To-Use (RTU) systems with inaccurate vendor recommended protocol settings, off-label use of RTU formats and less successful Abs in general (see Table 1).

Graph 1. **Proportion of sufficient results for SYP in the seventh NordiQC runs performed**



Conclusion

The mAb clones **27G12, BS15, DAK-SYNAP** and **Snp88** and the rmAb clones **MRQ-40** and **SP11** could all be used to obtain an optimal staining reaction for SYP. Irrespective of clone applied, HIER in an alkaline buffer was mandatory for an optimal staining reaction, and concentration of the primary Ab must be carefully calibrated. The RTU system GA660 (Dako/Agilent) for Omnis and based on the mAb clone DAK-SYNAP was the most successful assay obtaining a pass rate of 100%. The RTU systems 760-4595 and 790-4407 (Ventana/Roche), based on the rmAb MRQ-40 and SP11, respectively and being used by 33% provided a low pass rate using the vendor recommend protocol settings, but was found to be successful using modified protocol settings. The use of 3-step polymer/multimer based detection systems, regardless of assay type applied, provided the highest proportion of sufficient and optimal results.

Duodenum is the most recommendable positive tissue control for SYP. The axons of the Auerbach's and Meissner's plexus must display a high-level expression of SYP and the neuroendocrine cells of the mucosa and the peripheral nerves situated in lamina propria mucosa must show an at least weak to moderate staining reaction. Both the Paneth cells in the crypts of Lieberkuhn and the goblets cells must display an as strong as possible staining reaction. The goblets cells both the solid cytoplasmic compartment and mucin vesicles must be stained without any staining of the other epithelial cells.

Table 1. **Antibodies and assessment marks for SYP, Run 66**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **27G12**	50 2 1	Leica Biosystems Biocare Medical Monosan	20	19	12	2	74%	38%
mAb clone **DAK-SYNAP**	22	Dako/Agilent	12	5	5	-	77%	55%
mAb clone **UMAB112**	4	Origene	-	-	3	1	-	-
mAb clone **BS15**	3	Nordic Biosite	3	-	-	-	-	-
mAb clone **Snp88**	2	Biogenex	-	2	-	-	-	-
mAb clone **SY38**[5]	1	Dako/Agilent	1	-	-	-	-	-
mAb clone **ZM208**	1	Immunoforce	1	-	-	-	-	-
mAb clone **IHC669**	1	GenomeMe	1	-	-	-	-	-
rmAb clone **SP11**	5 2 1 1 1	Thermo/Neomarkers Abcam Spring Bioscience Epredia Zytomed	4	1	4	1	50%	40%
rmAb clone **MRQ-40**	5	Cell Marque	2	1	1	1	60%	40%
rmAb clone **EP158**	2	Cell Marque Quartett	1	1	-	-	-	-
rmAb clone **BP6053**	1	Biolynx	1	-	-	-	-	-
rmAb clone **GR007**	1	PathnSitu	-	-	1	-	-	-
Conc total	106		46	29	26	5	71%	43%

Ready-To-Use antibodies							Suff.[1]	OR.[2]
rmAb clone **SP11 790-4407**[3]	6	Ventana/Roche	-	-	2	4	0%	0%
rmAb clone **SP11 790-4407**[4]	66	Ventana/Roche	46	12	5	3	88%	70%
rmAb clone **MRQ-40 760-4595**[3]	4	Ventana/Roche	-	-	1	3	-	-
rmAb clone **MRQ-40 760-4595**[4]	51	Ventana/Roche	22	11	13	5	65%	43%
mAb clone **DAK-SYNAP IR/IS660**[3]	17	Dako/Agilent	3	7	7	-	59%	18%
mAb clone **DAK-SYNAP IR/IS660**[4]	21	Dako/Agilent	10	6	5	-	76%	48%
mAb clone **DAK-SYNAP GA660**[3]	34	Dako/Agilent	29	5	-	-	100%	85%
mAb clone **DAK-SYNAP GA660**[4]	29	Dako/Agilent	8	7	14	-	52%	28%
mAb clone **27G12 PA0299**[3]	12	Leica Biosystems	3	5	4	-	67%	25%
mAb clone **27G12 PA0299**[4]	12	Leica Biosystems	3	1	6	2	33%	25%
mAb clone **BS15 8453-C010**	2	Sakura Finetek	2	-	-	-	-	-
mAb clone **SNP88 AM363-10M**	2	BioGenex	1	1	-	-	-	-
mAb clone **MX038 MAB-0742**	1	Fuzhou Maixin	1	-	-	-	-	-
mAb clone **214A4G5 PA038**	1	Abcarta	-	-	1	-	-	-
mAb clone **27G12 OPAI371T60**	1	Biocare	-	-	-	1	-	-
mAb clone **C9D11 CSM-0250**	1	Celnovte	-	-	1	-	-	-
mAb clone **SYP02 MOB399-05**	1	Diagnostic Biosystems	-	-	1	-	-	-
rmAb clone **MRQ-40 336R-XX**	16	Cell Marque	5	4	3	4	56%	31%
rmAb clone **EP158 MAD-000685QD**	3	Master Diagnostica	3	-	-	-	-	-
rmAb clone **BP6053 l10412E**	2	Biolynx	2	-	-	-	-	-
rmAb clone **SP11 ab27601**	1	Abcam	-	1	-	-	-	-
pAb clone **336A-XX**	1	Cell Marque	-	-	1	-	-	-
RTU total	284		138	61	64	22	69%	49%
Total	390		184	90	90	27	-	
Proportion			47%	23%	23%	7%	70%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).
5) Clone terminated by vendor.

Detailed analysis of SYP, Run 66
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **27G12**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1; Ventana/Roche) (9/24)* or Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (11/20) as retrieval buffer. The mAb was typically diluted in the range of 1:30-1:140 depending on the total sensitivity of the protocol employed. Using these protocol settings, 32 of 38 (84%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer).

mAb clone **DAK-SYNAP**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (6/12), CC1 (Ventana/Roche) (5/7) or BERS2 (Leica Biosystems) (1/2) 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, 14 of 18 (88%) laboratories produced a sufficient staining result.

rmAb clone **SP11**: Protocols with optimal results were all based on HIER using CC1 (Ventana/Roche) (3/8) or Tris-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was diluted in the range of 1:50-1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings, 5 of 9 (56%) laboratories produced a sufficient staining result.

rmAb clone **MRQ-40**: Two protocols received an optimal result and was performed on the Benchmark Ultra platform (Ventana/Roche) using HIER in CC1 for 56 min. The rmAb was diluted 1:100 using OptiView as detection system.

mAb clone **BS15**: Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (1/1) (Dako/Agilent) or CC1 (Ventana/Roche) (2/2). The mAb was diluted 1:200-600 using a 3-step detection system. Using these protocol settings, 3 of 3 laboratories produced optimal staining results.

Table 2. **Proportion of optimal results for SYP for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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**	-	-	0/4**	-	9/24 (38%)	-	11/20 (55%)	0/3
mAb clone **DAK-SYNAP**	3/5 (60%)	0/1	3/7 (43%)	-	5/7 (71%)	-	1/2	-
rmAb clone **SP11**	-	-	-	-	3/8 (38%)	-	-	-
rmAb clone **MRQ-40**	-	-	-	-	2/4	-	0/1	-
mAb clone **BS15**	-	-	1/1	-	2/2	-	-	-

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

Ready-To-Use antibodies and corresponding systems
mAb clone **DAK-SYNAP**, product no. **IR660**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 97°C), 20-25 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 14 of 21 (67%) laboratories produced a sufficient staining result.

mAb clone **DAK-SYNAP**, product no. **GA660**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH 9 (GV804) (efficient heating time 30 min. at 97°C), 15-35 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 46 of 46 (100%) laboratories produced a sufficient staining result (all assessed as optimal).

rmAb clone **SP11**, product no. **790-4407**, Ventana/Roche, BenchMark XT, GX, ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 36-92 min.), 24-72 min. incubation of the primary Ab and UltraView (760-500) + amplification kit (760-080) or OptiView (760-700) +/- amplification (760-099/860-099) as detection systems. Using these protocol settings, 45 of 46 (98%) laboratories produced a sufficient staining result.

rmAb clone **MRQ-40**, product no. **760-4595**, Ventana/Roche, BenchMark XT, GX, ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-76 min.), 20-80 min. incubation of the primary Ab and UltraView (760-500) + amplification kit (760-080) or OptiView (760-700) +/- amplification (760-099/860-099) as detection systems. Using these protocol settings, 25 of 27 (93%) laboratories produced a sufficient staining result.

mAb clone **27G12**, product no. **PA0299**, Leica Biosystems, Bond III/MAX:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20-30 min. at 99-100°C), 15-24 min. incubation of the primary Ab and Bond Refine (DS9800) as detection system. Using these protocol settings, 10 of 17 (59%) laboratories produced a sufficient staining result (optimal or good).

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for SYP for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb DAK-SYNAP **IR660**	59% (10/17)	18% (3/17)	88% (8/9)	33% (3/9)
Dako Omnis mAb DAK-SYNAP **GA660**	100% (34/34)	85% (29/34)	52% (14/27)	26% (7/27)
Leica Bond III/MAX mAb 27G12 **PA0299**	67% (8/12)	25% (3/12)	30% (3/10)	20% (2/10)
VMS Ultra/XT rmAb SP11 **790-4407**	0% (0/6)	0% (0/6)	88% (58/66)	70% (46/66)
VMS Ultra/XT rmAb MRQ-40 **760-4595**	(0/4)	(0/4)	65% (33/51)	43% (22/51)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In concordance with the previous NordiQC assessments for SYP, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells and structures expected to be demonstrated. This pattern was observed in 100% of the insufficient results (117 of 117). Virtually all participating laboratories were able to stain SYP in cells with high-level expression as neoplastic cells of the neuroendocrine tumour (tissue core no. 5) and the neuroendocrine cells, ganglion cells and axons of the nerve plexus in the duodenum. Demonstration of SYP in paneth cells of the duodenum crypts, goblets cells, and/or the neoplastic cells of the small cell lung carcinoma was more challenging and could only be demonstrated when applying optimized and appropriate protocol settings.

Concentrated formats were used by 27% (106/390) of the participants in this seventh SYP assessment compared to 40% (124/308) in the last run 52, 2018. The general pass-rate for participants using the concentrated formats was 71% (75 of 106), 43% (46 of 106) being optimal.

The mAb clone 27G12 was the most widely used concentrated primary antibody for the demonstration of SYP and was used by 53 laboratories within a laboratory developed (LD) assay. Optimal results were only seen using the Ventana Benchmark or the Leica Bond systems in a high concentration (1:30-140) and only with 3-layer detection systems. 4 participants applied the concentrate on the Dako Omnis, but no optimal results observed, despite concentrate was applied with similar central protocol settings as the ones used on both Ventana and Bond platforms. Insufficient protocols were typically caused by a too high dilution of

the primary antibody, too short HIER and/or use of 2-layer detection system which of central importance resulted in an almost negative staining of the neoplastic cells of the SCLC.

The mAb clone DAK-SYNAP used within a LD-assay, gave an overall pass rate of 77% (17 of 22) of which 55% (12 of 22) of the results were assessed as optimal. The mAb clone DAK-SYNAP was the only clone in this assessment that provided optimal results on all four main IHC platforms (see Table 2). As for the mAb clone 27G12, identical technical parameters influenced the performance of the mAb DAK-SYNAP and therefore, it is recommendable to use a sensitive 3-step detection system and carefully calibrate the titer of the primary Ab in relation to the critical staining indicators (e.g. the goblet cells in duodenum epithelium for analytical sensitivity and e.g. smooth muscle cells for basic analytical specificity).

The rmAb clone SP11 used within a LD-assay, gave an overall pass rate of 50% (5 of 10) of which 40% of the results (4 of 10) were assessed as optimal. The Ab was only applied on the BenchMark IHC platform (Ventana/Roche) and thus not possible to evaluate the robustness and performance of the Ab on the other main IHC platforms.
The rmAb MRQ-40 was used by 5 participants with a pass-rate of 60% (3 of 5).
For both clones MRQ-40 and SP11, no significant technical parameters could be identified distinguishing insufficient from sufficient results. However, similar to the mAb clones 27G12 and DAK-SYNAP protocols based on efficient HIER in an alkaline buffer in combination with 3-layer detection systems were most successful.
The mAb clone BS15 was only used by 3 laboratories as a concentrated format but all with optimal results. The dilution factor for this clone was higher (1:200-600) compared to the other clones applied within a LD assay, but still with a 3-layer detection system. The staining pattern for this clone was a bit different than observed with the clones 27G12, DAK-SYNAP, SP11 and MRQ-40. It was observed that the clones BS15 (and clones BP6053 and MX038) provided an enhanced staining reaction in the SCLC labelling more cells with increased intensity compared to the other clones listed above.

73% (284/390) of the laboratories used a Ready-To-Use (RTU) system for SYP, being increased from 60% in the last run. The pass-rate among the participants using RTU products was 69% (198 of 284) of which 49% of the results were assessed as optimal.
In this assessment, the RTU system GA660 based on mAb clone DAK-SYNAP (Dako/Agilent) applied by the vendor recommended protocol settings was the most successful assay for the demonstration of SYP providing a pass rate of 100% (34 of 34), 85% (29 of 34) being optimal. The vendor recommended protocol was based on HIER using TRS high for 30 min., Ab incubation for 25 min. and the FLEX+ protocol (GV800/GV823+GV821). 13 laboratories modified the protocol by removing the linker, all provided an insufficient result. 2 participants used the RTU format off-label on a non-intended IHC platform.
The Dako/Agilent IR/IS660 RTU system for Autostainer was applied by 38 participants. 17 were using the vendor recommended protocol settings with HIER in TRS high for 20 min., Ab incubation for 20 min. and EnVision FLEX as detection system, giving a pass-rate of 59% (10 of 17), but only 18% (3/17) being optimal results. The main reason for insufficient results was due to a too weak staining of cells expected to be demonstrated, and was seen in 62% (16 of 26) of the insufficient cases.
Laboratory protocol modifications applying linker (FLEX+) was found to be very successful giving a pass-rate of 100% (4 of 4) indicating that the vendor recommendations might be changed to using the FLEX+ system (K8000/K8002).
12 laboratories used the IR/IS660 product on non-intended platforms with mixed results.

The Ventana/Roche RTU system based on rmAb clone SP11 (790-4407) was used by 72 participants in total. Applying the vendor recommended protocol settings an overall pass rate of 0% (0 of 6) was provided. The vendor protocol recommendations indicate UltraView as detection system in combination with mild CC1 for HIER and an incubation time of the primary antibody for 32 min. In contrast, the overall pass-rate was 88% (58 of 66) of which 70% (46 of 66) being optimal, when modified protocol settings were applied. The most successful change was related to usage of a 3-layer detection system either OptiView (760-700) or UltraView (760-500) with amplification kit (760-080) and with these settings 95% (56 of 59) of the protocols submitted generated sufficient results, indicating it would be beneficial for the overall performance of the RTU system based on the rmAb clone SP11 if the vendor changed the protocol recommendations to a more sensitive detection system.

The Ventana RTU system for the BenchMark IHC platform based on rmAb clone MRQ-40 (760-4595) was in total used by 55 participants. Only 4 applied the RTU system as suggested by vendor using UltraView as detection system in combination with mild CC1 for HIER and an incubation time of 16 min. for the primary antibody. These settings gave an overall pass rate of 0% similar to the RTU system based on the clone SP11. This observation and performance was also seen in the previous assessment run 52.

The vast majority of participants modified the protocol settings for the Ventana/Roche MRQ-40 based RTU system giving an overall pass rate of 65%, 43% optimal. Especially the exchange of UltraView to UltraView + amplification or OptiView as detection system was found successful providing a pass rate of 91% (31/34), 62% (21/34) being optimal. In comparison, UltraView without amplification as the detection system gave a significantly inferior pass rate of only 10% (2/20), 5% (1/20) optimal. In addition, prolonged HIER and primary Ab incubation time was also found successful, except for protocols based on OptiView with amplification.

The Leica Biosystems RTU system PA0299 based on mAb clone 27G12 was used by 24 laboratories. Two laboratories applied it on a non-intended IHC platform. The vendor recommended protocol setting was based on HIER in BERS2 pH 9 for 20 min. and protocol F (15 min. incubation time of the primary Ab and a 3-layer detection system). In the last run 52 this RTU system did not provide any optimal results either by the vendor recommended or modified protocol settings and the pass-rate was only 46%. In this run the pass rate increased to 67% (8 of 12) and 25% (3 of 12) optimal results, when the RTU system was applied in compliance with the recommended protocol settings. The lack of analytical accuracy using optimal protocol settings could not be explained and related to e.g. lot. number and the reason for the high variation is still unclear.

This was the seventh assessment of SYP in NordiQC and over the last two runs the pass-rate has steadily declined (see Graph 1). During the last two assessments the number of participants has increased immensely and in this assessment, we have also observed a change from concentrated formats to Ready-to-Use products. The reduced pass rate was primarily due to extended use of Ready-To-Use (RTU) systems with inaccurate vendor recommended protocol settings, providing an overall too low analytical sensitivity in the materials used for the assessment.
Only the Dako/Agilent RTU system GA660 based on the clone DAK-SYNAP for the Dako Omnis was highly successful as "plug-and-play, whereas the other widely used RTU systems typically required altered protocol settings for the expected performance and level of analytical sensitivity. It was observed that new RTU systems based on the clones BS15, BP6053 and MX038 showed promising results although the data was limited

Controls
Duodenum is recommended as positive and negative tissue control for the detection of SYP. The protocol must be calibrated to give an intense staining reaction of the axons of the Auerbach's and Meissner's plexus with a high-level expression of SYP. The neuroendocrine cells of the mucosa and the peripheral nerves situated in lamina propria mucosa must show an at least weak to moderate staining reaction. The Paneth cells and the goblet cells both the solid cytoplasmic compartment and mucin vesicles must display an as strong as possible staining reaction without any staining of the other epithelia cells. Furthermore, it was observed that the goblet cells at the luminal surface showed an increased staining intensity compared to the cells at the basal part of the Lieberkühn crypts. In optimal protocols typically all goblet cells were stained also in the bottom of the crypts.

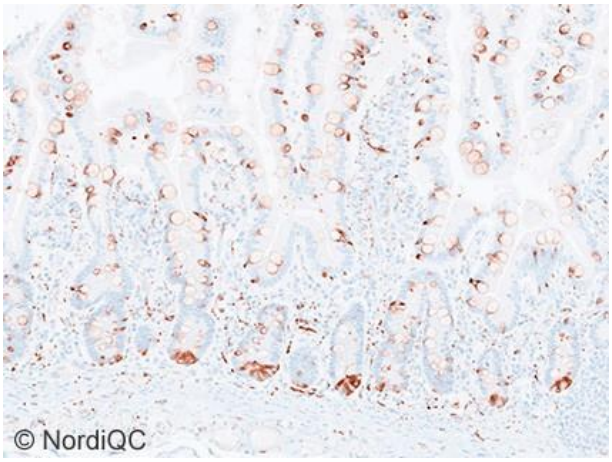


Fig. 1a (x100)
Optimal staining reaction for SYP in the duodenum using the rmAb clone SP11 (RTU 790-4407, Ventana/Roche), HIER in CC1 (64 min.) and OptiView (3-step multimer) as detection system. The Paneth cells in the bottom of the crypt of Lieberkühn display a moderate to strong staining reaction. The goblet cells both in the mucin contained in the cytoplasm and in the cell membrane

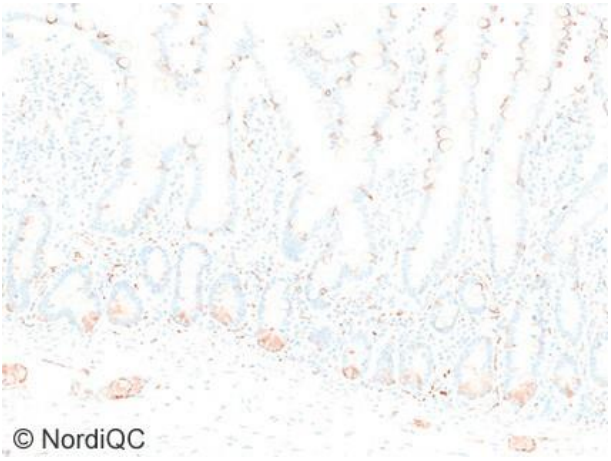


Fig. 1b (x100)
Insufficient staining reaction for SYP in the duodenum using the rmAb clone MRQ-40 (RTU 760-4595, Ventana/Roche), HIER in CC1 (64 min.) and UltraView (2-step multimer) as detection system. The Paneth cells in the bottom of the crypt of Lieberkühn display a weak to moderate staining reaction and many of the goblet cells in the bottom of the crypts are completely negative.

surrounding the mucin display a moderate to strong staining reaction without any staining of the other epithelial cells. Same protocol used in Figs. 2a-4a.

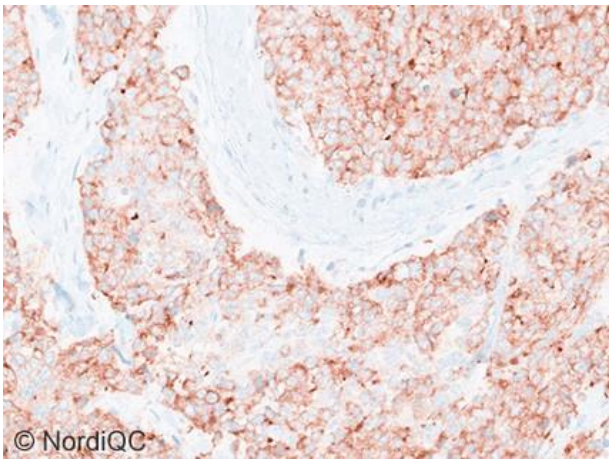


Fig. 2a (x200)
Optimal staining reaction of SYP in the SCLC using same protocol as in Figs. 1a-4a. Virtually all neoplastic cells show a moderate to strong and distinct staining reaction. No background staining is seen.

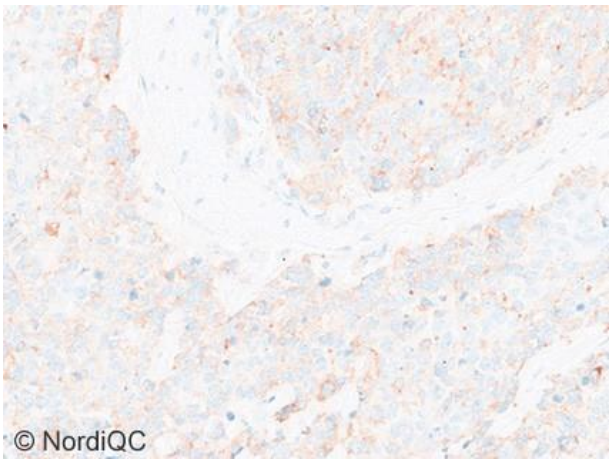


Fig. 2b (x200)
Insufficient staining reaction of SYP in the SCLC using same protocol as in Figs. 1b-4b - same field as in Fig. 2a. The neoplastic cells display a too weak staining intensity and a reduced number of cells are demonstrated.

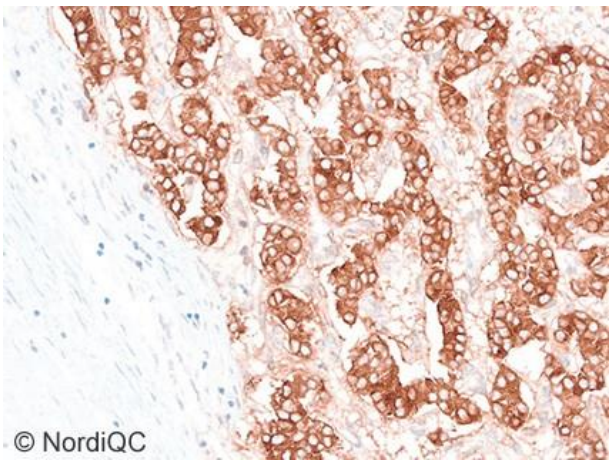


Fig. 3a (x200)
Optimal staining reaction of SYP in the neuroendocrine tumour using same protocol as in Figs. 1a-4a. All neoplastic cells are strongly stained.

Same protocol used in Figs 2b-4b – same field as 1a.

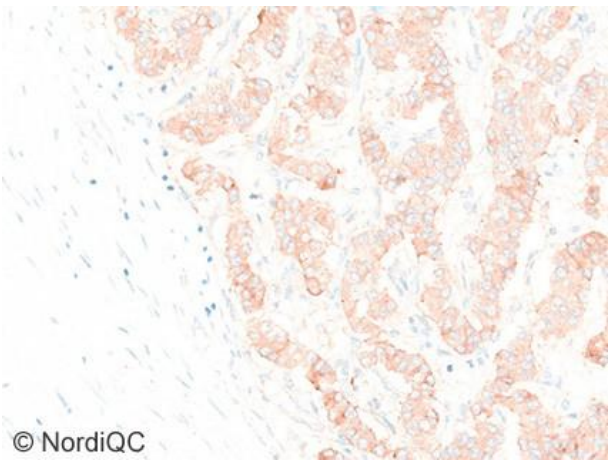


Fig. 3b (x200)
Staining reaction of SYP in the neuroendocrine tumour – same field as in Fig. 3a. Although the neoplastic cells display moderate staining intensity, the protocol provides an overall too low sensitivity – compare with Fig. 1a - 4b.

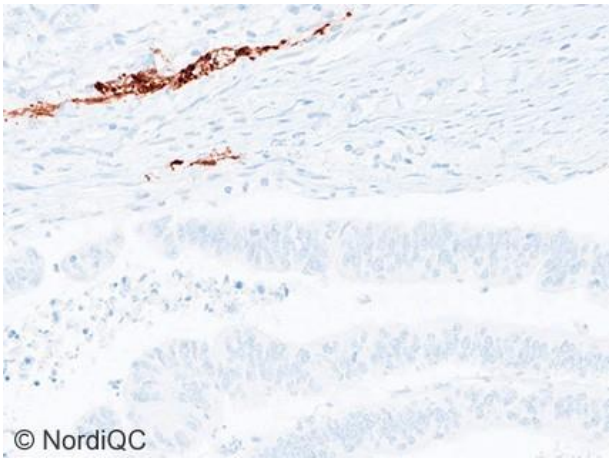


Fig. 4a (x200)
Optimal staining reaction of SYP in the colon adenocarcinoma using same protocol as in Figs. 1a - 3a. As expected, no staining of neoplastic cells is seen. The peripheral nerves in the stromal compartment show a strong and distinct cytoplasmic staining reaction.

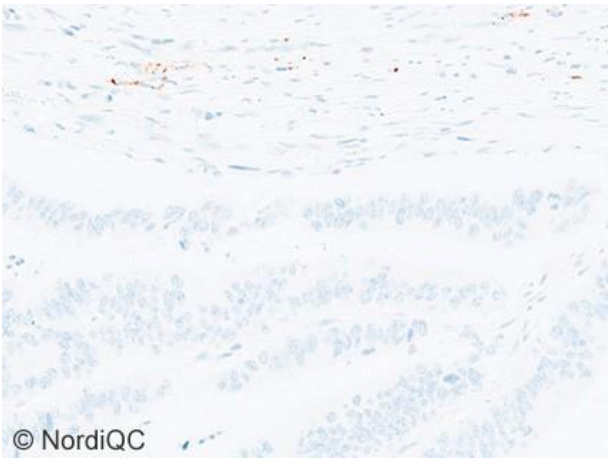


Fig. 4b (x200)
Insufficient staining reaction of SYP in the colon adenocarcinoma using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The neoplastic cells are negative, but the peripheral nerves in the stromal compartment only display a faint to weak staining reaction.

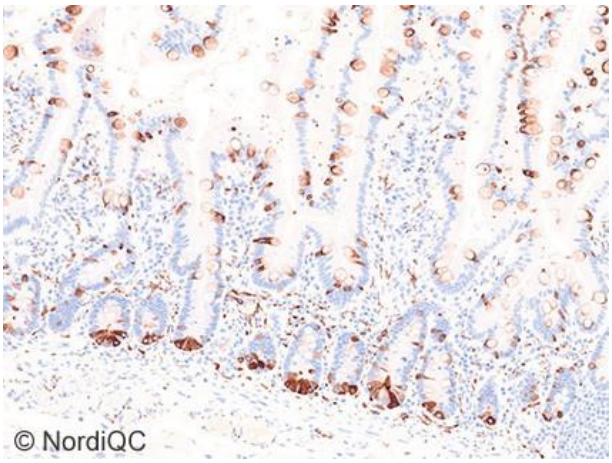


Fig. 5a (x100)
Optimal staining reaction for SYP in the duodenum using the mAb clone DAK-SYNAP (RTU GA660, Dako/Agilent), with vendor recommended protocol settings. The Paneth cells in the bottom of the crypt of Lieberkühn display a strong staining reaction. The goblet cells both in the mucin contained in the cytoplasm and in the cell membrane surrounding the mucin display a moderate to strong staining reaction without any staining of the other epithelia cells. Same protocol used in Fig. 6a.

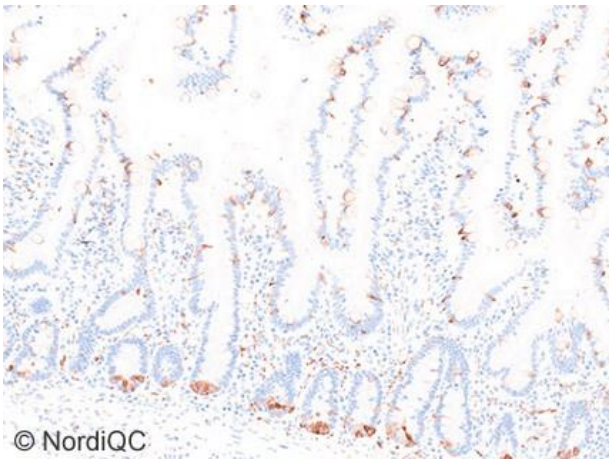


Fig. 5b (x100)
Insufficient staining reaction for SYP in the duodenum using the mAb clone DAK-SYNAP (RTU GA660, Dako/Agilent), with the same settings as in Fig. 5a but without linker. The Paneth cells in the bottom of the crypt of Lieberkühn display a weak to moderate staining reaction and only few goblet cells in the bottom of the crypts are weakly stained.
Same protocol used in Fig. 6b – same field as 1a- 1b and 5a.

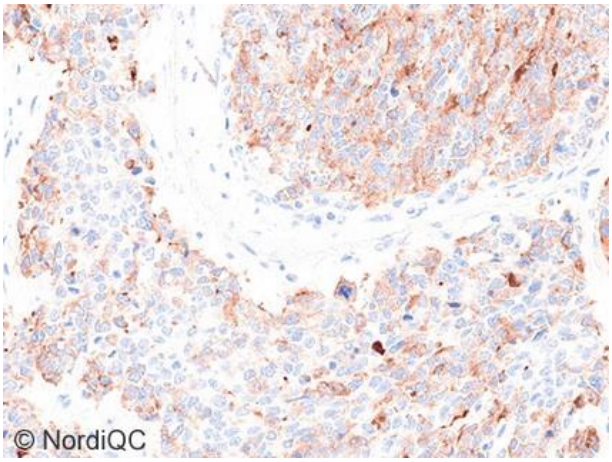


Fig. 6a (x200)
Optimal staining reaction of SYP in the SCLC using same protocol as in Fig. 5a. All majority of the neoplastic cells show a weak to strong cytoplasmic staining reaction.

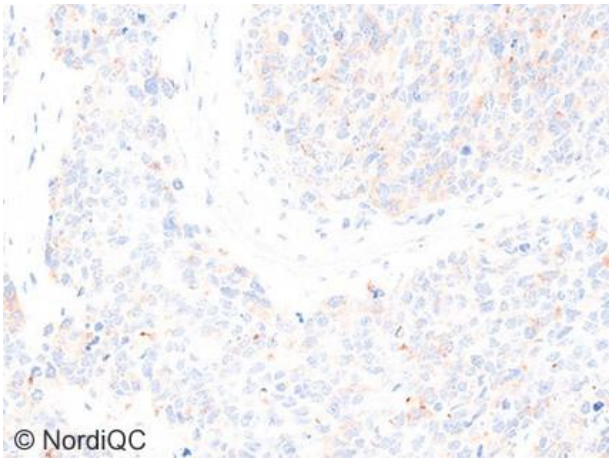


Fig. 6b (x200)
Insufficient staining reaction of SYP in the SCLC using same protocol as in Fig. 5b – same field as Figs. 2a- 2b and 6a. The neoplastic cells display a too weak staining intensity with large parts of the tumor completely negative.

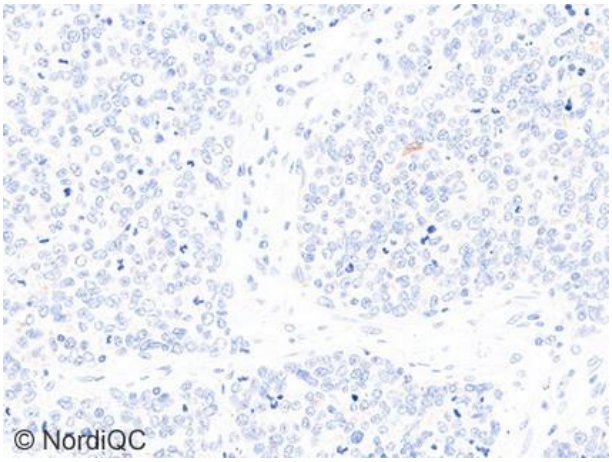


Fig. 7a (x200)
Insufficient staining of SYP in the SCLC
using the mAb clone 27G12 (RTU PA0299, Leica Biosystems), with a laboratory modified protocol based on HIER in BERS1 for 10 min. The tumour is completely negative. Also see Fig. 7b, same protocol.

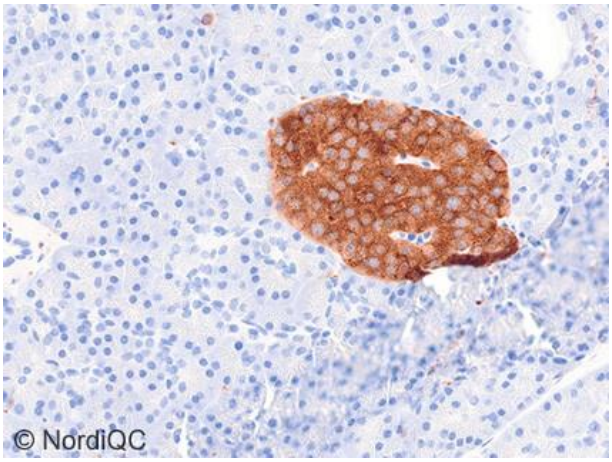


Fig. 7b (x200)
Laboratory On-slide control of a pancreas using same protocol settings as in Fig. 7a. Virtually all endocrine islet cells show a strong and distinct cytoplasmic staining reaction, but the peripheral nerve fibres are almost completely negative. The high level expression of SYP in the endocrine cells comprises the utility of pancreas as positive tissue control for SYP to evaluate the critical level of analytical sensitivity.

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