Assessment Run 73 2025

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

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].
- 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.

KEY POINTS FOR SYP IMMUNOASSAYS
- 3-step detection systems are crusial for optimal performance
- The mAb clone 27G12 both as concentrate and RTU system was less successful
- The mAb clone DAK-SYNAP both as a concentrate and RTU-system displayed a high pass-rate
- Duodenum is recommended as positive and negative tissue control for the demonstration of SYP.

Participation

Number of laboratories registered for SYP, run 73	445
Number of laboratories returning slides	430 (97%)

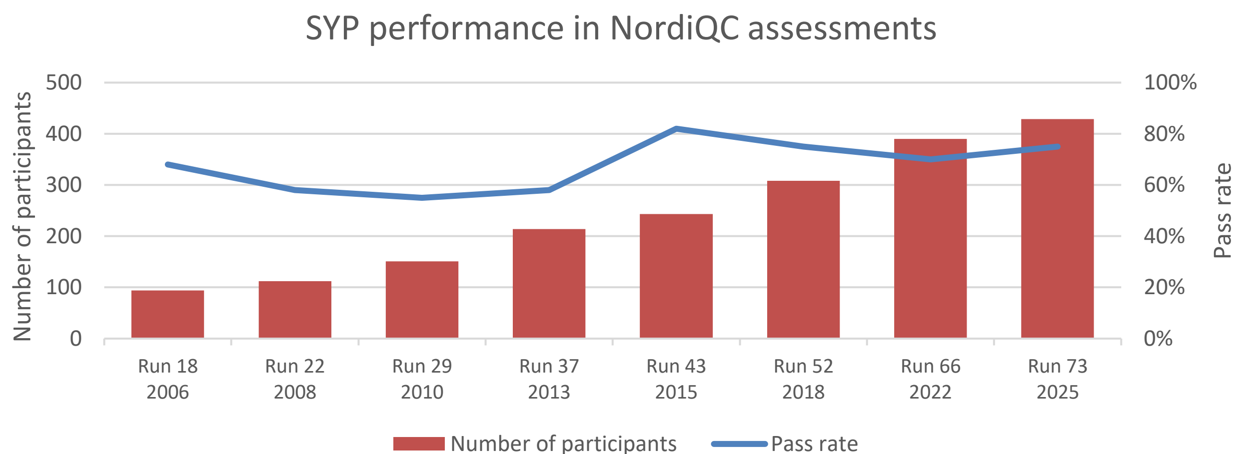
Results
At the date of assessment, 97% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report. One slide was received broken and was not scored (the slide could not be mended).

429 laboratories participated in this assessment and 75% achieved a sufficient mark (optimal or good). Table 1a, b and c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining were:
- Inefficient HIER – too short time or use of 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.
- Less successful clones 27G12 and MRQ-40

Performance history
This was the eighth NordiQC assessment of SYP. A slightly increased pass rate was observed compared to the previous run 66 and on par to the level seen in run 58 (see Graph 1). The improved pass rate was primarily due to extended use of 3-layer detection systems and robust RTU systems.

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



Controls
Duodenum is recommended as positive and negative tissue control for the demonstration 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. In the Paneth and 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.

Conclusion
The mAb clones **27G12, BS15, DAK-SYNAP** and the rmAb clones **MRQ-40, SP11, ZR445** and **QR054** 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% and 100% optimal results.
The RTU system PA0299 (Leica Biosystems), based on the mAb clone 27G12 being used by 12% (41 of 353) provided a low pass rate using the vendor recommend protocol settings and also as modified protocols. The use of 3-step polymer/multimer based detection systems, regardless of assay type applied, provided the highest proportion of sufficient and optimal results.

Table 1a. **Overall results for SYP, run 73**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	76	41	17	18	-	76%	54%
Ready-To-Use antibodies	353	198	66	81	8	75%	56%
Total	429	239	83	99	8		
Proportion		56%	19%	23%	2%	75%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for SYP, run 73**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **27G12**	36	Leica Biosystems	15	10	11	-	69%	42%
mAb clone **DAK-SYNAP**	20	Dako/Agilent	14	5	1	-	95%	70%
mAb clone **BS15**	4	Nordic Biosite	4	-	-	-	-	-
mAb clone **ZM208**	1	Zeta Corporation	1	-	-	-	-	-
rmAb clone **SP11**	2 1 1	Thermo/Neomarkers Abcam Diagnostic BioSystems	2	-	2	-	-	-
rmAb clone **MRQ-40**	7	Cell Marque	3	1	3	-	57%	43%
rmAb clone **EP158**	2	Master Diagnostica Bio SB	-	1	1	-	-	-
rmAb clone **ZR445**	1	Zeta corporation	1	-	-	-	-	-
rmAb clone **QR054**	1	Quartett	1	-	-	-	-	-
Total	76		41	17	18	-		
Proportion			54%	22%	24%	0%	76%	

1) Proportion of sufficient stains (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results.

Table 1c. **Ready-To-Use antibodies and assessment marks for SYP, run 73**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **SP11 OptiView 790-4407**[3]	8	Ventana/Roche	6	2	-	-	100%	75%
rmAb clone **SP11 UltraView 790-4407**[3]	5	Ventana/Roche	3	2	-	-	100%	60%
rmAb clone **SP11 OptiView 790-4407**[4]	67	Ventana/Roche	43	19	5	-	93%	64%
rmAb clone **SP11 UltraView 790-4407**[4]	24	Ventana/Roche	11	2	9	2	54%	46%
rmAb clone **MRQ-40 760-4595**[3]	4	Ventana/Roche	-	-	2	2	-	-
rmAb clone **MRQ-40 760-4595**[4]	57	Ventana/Roche	23	15	19	-	67%	40%
mAb clone **DAK-SYNAP GA660**[3]	59	Dako/Agilent	59	-	-	-	100%	100%
mAb clone **DAK-SYNAP GA660**[4]	28	Dako/Agilent	20	5	3	-	89%	71%
mAb clone **DAK-SYNAP IR/IS660**[3]	11	Dako/Agilent	7	1	3	-	73%	64%
mAb clone **DAK-SYNAP IR/IS660**[4]	20	Dako/Agilent	11	7	2	-	90%	55%

mAb clone **27G12** **PA0299**[3]	25	Leica Biosystems	-	5	20	-	20%	0%
mAb clone **27G12** **PA0299**[4]	16	Leica Biosystems	1	4	10	1	31%	6%
rmAb clone **MRQ-40** **336R-XX**	14	Cell Marque	5	2	4	3	50%	36%
mAb clone **MX038** **MAB-0742**	1	Fuzhou Maixin	1	-	-	-	-	-
mAb clone **214A4G5** **PA038**	1	Abcarta	-	-	1	-	-	-
mAb clone **27G12** **IP371**	1	Biocare Medical	-	-	1	-	-	-
mAb clone **C9D11** **CSM-0250**	1	Celnovte	1	-	-	-	-	-
mAb clone **SP11** **RMPD018**	1	Diagnostic Biosystems	-	1	-	-	-	-
mAb clone **BS15** **8453-C010**	3	Sakura Finetek	3	-	-	-	-	-
rmAb clone **EP158** **MAD-000685QD**	1	Master Diagnostica	-	-	1	-	-	-
rmAb clone **EP158** **MAD-000685QD**	1	Vitro SA	1	-	-	-	-	-
rmAb clone **BP6053** **l10412E**	1	Biolynx Biotechnology	1	-	-	-	-	-
pAb clone **336A-XX**	1	Cell Marque	-	1	-	-	-	-
Clone **GR306** **GT253402**	1	Gene Tech	1	-	-	-	-	-
rmAb clone **BY023** **BFM-0311**	1	Bioin Biotechnology	1	-	-	-	-	-
Clone **DY49905** **4920062**	1	Dakewe	-	-	1	-	-	-
Total	353		198	66	81	8		
Proportion			56%	19%	23%	2%	75%	

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 assessed 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 assessed protocols)

Detailed analysis of SYP, Run 73
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) (9/17)* or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (6/16) 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, 23 of 29 (79%) 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) (1/1), TRS pH 9 (Dako/Agilent) (4/6), CC1 (Ventana/Roche) (8/8) 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, 16 of 17 (94%) laboratories produced a sufficient staining result.

rmAb clone **MRQ-40**: Three protocols received an optimal result and was performed on either the Benchmark Ultra platform (Ventana/Roche) using HIER in CC1 for 64 min. or on the Autostainer platform (Dako/Agilent) using TRS pH 9 (3-in-1) for 20 min. The rmAb was diluted 1:25-50 using a 3-layer detection system.

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

Concentrated antibody	Dako/Agilent Autostainer[1]		Dako/Agilent Omnis		Ventana/Roche BenchMark[2]		Leica Biosystems Bond[3]	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **27G12**	-	-	0/1	-	53% (9/17)	-	38% (6/16)	0/2
mAb clone **DAK-SYNAP**	1/1	0/1	67% (4/6)	-	100% (8/8)	-	1/2	0/1
rmAb clone **MRQ-40**	1/1	-	-	-	33% (2/6)	-	-	-

* 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)
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra Plus
3) Bond III, Prime, Max

Ready-To-Use antibodies and corresponding systems

mAb clone **DAK-SYNAP**, product no. **IR/IS660**, 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 20-40 min. at 97°C), 10-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection systems. Using these protocol settings, 16 of 20 (80%) laboratories produced a sufficient staining result.
9 laboratory used product no. IR/IS660 for staining on another platform. Data was not included in the description above

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 20-30 min. at 97°C), 20-35 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 73 of 73 (100%) laboratories produced a sufficient staining result (all assessed as optimal).
2 laboratory used product no. GA660 for staining on another platform. Data was not included in the description above

rmAb clone **SP11**, product no. **790-4407**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-92 min.), 24-72 min. incubation of the primary Ab and UltraView (760-500) primarily with amplification kit (760-080) or OptiView (760-700) +/- amplification (760-099/860-099) as detection systems. Using these protocol settings, 80 of 91 (88%) laboratories produced a sufficient staining result.

rmAb clone **MRQ-40**, product no. **760-4595**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-76 min.), 16-80 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, 26 of 34 (76%) laboratories produced a sufficient staining result.

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 **IR/IS660**	73% (8/11)	64% (7/11)	91% (10/11)	55% (6/11)
Dako Omnis mAb DAK-SYNAP **GA660**	100% (59/59)	100% (59/59)	89% (23/26)	69% (18/26)
Leica Bond mAb 27G12 **PA0299**	20% (5/25)	0% (0/25)	21% (3/14)	0% (0/14)
Ventana BenchMark rmAb SP11 **790-4407**	100% (13/13)	69% (9/13)	82% (75/91)	59% (54/91)
Ventana BenchMark rmAb MRQ-40 **760-4595**	(0/4)	(0/4)	67% (38/57)	40% (23/57)

* 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 are 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 90% of the insufficient results (96 of 107). The remaining causes of insufficient results were due to either poor signal-to-noise ratio or excessive background.
Virtually all participating laboratories were able to demonstrate SYP in cells with high-level expression as neoplastic cells of the neuroendocrine tumour (tissue core no. 3), 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 18% (76/429) of the participants in this eighth SYP assessment compared to 27% (106 of 390) in the last run 66, 2022. The general pass-rate for participants using the concentrated formats was 76% (58 of 76), 54% (41 of 76) being optimal.

The mAb clone **27G12** was the most widely used concentrated primary antibody for the demonstration of SYP and was used by 36 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 (1:50-100), HIER in an alkaline buffer and only with 3-layer detection systems. One of the participants applied the concentrate on the Dako Omnis and despite the concentration being applied with similar central protocol settings as the ones used on both Ventana and Bond platforms, the total sensitivity of the protocol gave a too weak result. These observations have been seen in earlier assessments indicating that the 27G12 clone is challenging, especially on the Dako Omnis platform.
The mAb clone 27G12 was only acquired from Leica Biosystems in this assessment where the package insert suggests protocol settings using a dilution of 1:200 and low pH as retrieval buffer. These settings did not produce any sufficient results. Pooling both concentrated formats and Ready-to-use products (n=5) no sufficient results were produced using HIER in a low pH buffer.
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 result of the neoplastic cells of the SCLC.

The mAb clone **DAK-SYNAP** used within a LD-assay, gave an overall pass rate of 95% (19 of 20) of which 70% (14 of 20) 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 **MRQ-40** was used by 7 participants with a pass-rate of 57% (4 of 7).
For MRQ-40 optimal results were produced by using the primary Ab in high concentrations 1:25-50, HIER in an alkaline buffer and a 3-layer detection system.

The mAb clone **BS15** was only used by 4 laboratories as a concentrated format but all with optimal results. The dilution factor for this clone was higher (1:400-1.500) 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 in general 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 (See Figs. 5a-b).

82% (353 of 429) of the laboratories used a Ready-To-Use (RTU) system for SYP, being increased from 73% in the last run. The pass-rate among the participants using RTU products was 75% (264 of 353) of which 56% of the results were assessed as optimal.

In this assessment, the RTU system **GA660** based on mAb clone DAK-SYNAP for the Omnis platform (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% (59 of 59), all 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). 26 laboratories modified the protocol on Omnis and obtained a pass rate of 89%, 69% optimal. 8 laboratories lowered the analytical sensitivity by either removing the linker or shortening incubation times. Modifications like this resulted in a too weak staining result, which in some cases affected the interpretation.

The Dako/Agilent **IR/IS660** RTU system for Autostainer was applied by 31 participants. 11 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 73% (8 of 11), 64% (7/11) 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 80% (4 of 5) of the insufficient cases.
Laboratory modifications involving the application of linker (FLEX+) were highly successful, achieving a pass rate of 100% (6 out of 6). However, it was noted that two laboratories experienced excessive background due to an excessively sensitive protocol. In contrast, the remaining four laboratories adjusted the primary antibody incubation when incorporating the linker, producing optimal results. These findings suggest that modifications should be implemented with careful calibration to ensure accuracy and precision. 9 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 104 participants in total. Applying the vendor recommended protocol settings an overall pass rate of 100% (13 of 13) was provided which is in contrast to the latest run 66 where the pass-rate was 0%. The vendor protocol recommendations have been updated since the latest run 66, where only UltraView as detection system were recommended. Now the recommendations include both OptiView and UltraView and the UltraView protocol has since been carefully calibrated to provide an improved performance as shown in the results (see table 1c and 3). The UltraView protocol has been updated using HIER in CC1 for 52 min. and an antibody incubation time of 72 min. The OptiView protocol indicate HIER in CC1 for 40 min. and a primary Ab incubation of 36 min. With these new settings the vendor recommended protocols displayed very similar pass-rates for the two detection systems applied.
Laboratory modifications resulted in a slightly reduced pass-rate when using OptiView compared to vendor recommendations. The primary issues were laboratories either shortening antibody incubation times, leading to weaker staining, or adding amplification, causing excessive background or granular staining that compromised the interpretation.
Using UltraView as detection system 12 laboratories applied amplification with a pass-rate of 92% (11 of 12). Using UltraView as stand-alone with shortened incubations times compared to vendor recommendations the pass-rate was only 17% (2/12).

The Ventana RTU system for the BenchMark IHC platform based on rmAb clone MRQ-40 (**760-4595**) was in total used by 61 participants. Only 4 applied the RTU system as suggested by vendor recommended settings using UltraView as detection system in combination with HIER in CC1 for 32 min. and an incubation time of 16 min. for the primary antibody. These settings gave an overall pass rate of 0%. This observation and performance was also seen in the two previous assessment runs 52 and 66.
The vast majority of participants modified the protocol settings for the Ventana/Roche MRQ-40 based RTU system giving an overall pass rate of 67%, 40% optimal. Especially the exchange of UltraView to UltraView + amplification or OptiView as detection system was found successful providing a pass rate of 80% (36 of

45), 48% (22 of 45) being optimal. In comparison, UltraView without amplification as the detection system gave a significantly inferior pass rate of only 12% (2 of 16), 6% (1 of 16) 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** for the Bond IHC platforms based on mAb clone 27G12 was used by 41 laboratories. Two laboratories applied it on a non-intended IHC platform. The vendor recommended protocol setting is based on HIER in BERS2 pH 9 for 20 min. and protocol F (15 min. incubation time of the primary Ab with a 3-layer detection system). In run 52, the RTU system achieved a pass-rate of only 46% with neither vendor nor modified protocols producing optimal results. Run 66 showed some improvement in the pass-rate to 67%. However, in this current run a very low pass-rate was observed. This was seen for both protocols based on vendor recommended and modified settings giving pass rates of 20% (5/25 participants) and 21% (3 of 14 participants), respectively and no optimal results.

Despite the PA0299 product performing poorly over the last two runs, the number of participants using this product has almost doubled from the previous run to the current one. Due to the inferior performance of the RTU system PA0299, laboratories are highly encouraged to verify the performance of this IHC assay and evaluate the diagnostic accuracy for SYP demonstration. From the NordiQC data generated it seems to be necessary to recalibrate the protocol for the RTU assay or change to the concentrated format of the clone 27G12 or other clone and revalidate the IHC assay for SYP.

The eighth assessment of SYP in NordiQC showed an improvement in the pass-rate, increasing from 70% to 75% since run 66. This progress is attributed to an extended use of 3-layer detection systems and more robust RTU systems available.
The number of participants has grown significantly over the last three assessments, and a large shift from concentrated formats to Ready-to-Use (RTU) products has been observed.
The Dako/Agilent RTU system GA660, based on the clone DAK-SYNAP for the Dako Omnis, was highly successful as a true "plug-and-play" system giving a pass rate of 100%. The number of participants using this system has increased from 63 participants in the latest run 66 to 87 participants and this had a positive impact on the overall pass rate.
A major improvement was also observed with the Ventana/Roche's updated protocol recommendations for the RTU system based on the rmAb clone SP11 790-4407, marking a significant improvement for both OptiView and UltraView based protocols.
However, the performance of the 27G12 clone and the MRQ-40 negatively impacted the overall pass-rate, as both clones seems very challeging.
Despite limited data RTU systems based on clones BS15, BP6053 and MX038 showed promising results.

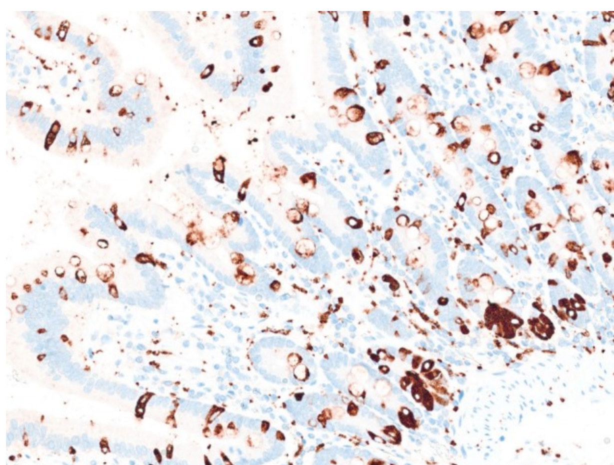


Fig. 1a (x200)
Optimal staining reaction for SYP in the duodenum using the rmAb clone SP11 (RTU 790-4407, Ventana/Roche), HIER in CC1 (64 min.) and OptiView (3-step multimer) as detection system. The Paneth cells in the bottom of the crypt of Lieberkühn display a moderate to strong staining reaction. The goblet cells both in the mucin in the cytoplasmic compartment and in the cell membrane surrounding the mucin display a moderate to strong staining reaction without any staining reaction of the other epithelial cells. Same protocol used in Figs. 2a-4a.

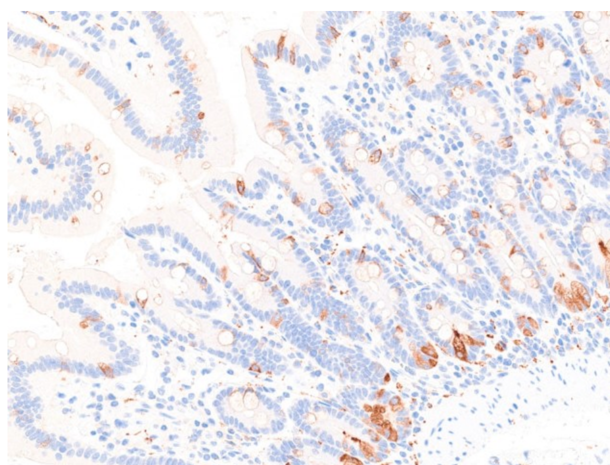


Fig. 1b (x200)
Insufficient staining reaction for SYP in the duodenum using the mAb clone 27G12 (RTU PA0299, Leica Biosystems), HIER in BERS2 (30 min.) and Bond Refine (3-step polymer) as detection system. The Paneth cells in the bottom of the crypt of Lieberkühn display a weak to moderate staining reaction and many of the goblet cells in the bottom of the crypts are completely negative. Same protocol used in Figs 2b-4b – same field as 1a.

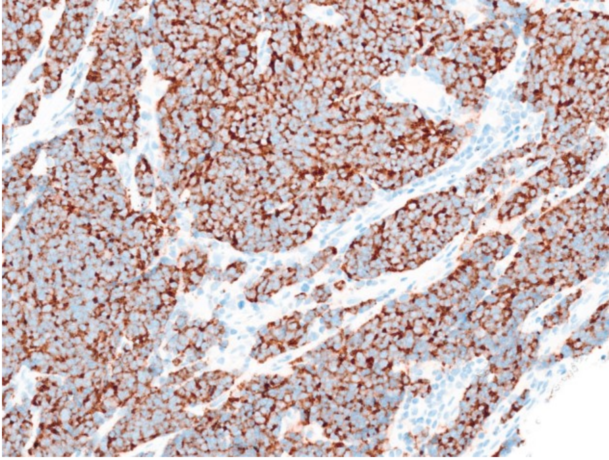


Fig. 2a (x200)
Optimal staining reaction of SYP in the SCLC using same protocol as in Figs. 1a-4a. Virtually all neoplastic cells show a moderate to strong and distinct staining reaction. No background staining is seen.

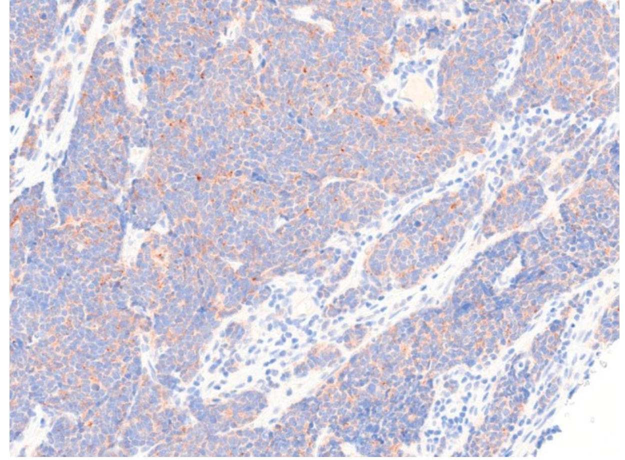


Fig. 2b (x200)
Insufficient staining reaction of SYP in the SCLC using same protocol as in Figs. 1b-4b - same field as in Fig. 2a. The neoplastic cells display a too weak staining intensity and a reduced number of cells are demonstrated.

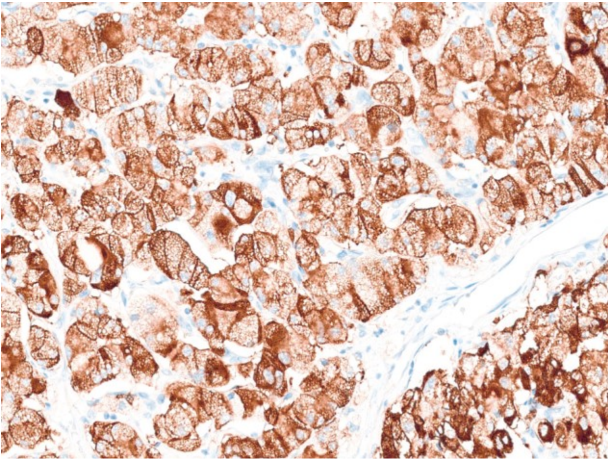


Fig. 3a (x200)
Optimal staining reaction of SYP in the neuroendocrine carcinoma using same protocol as in Figs. 1a-4a. All neoplastic cells show a distinct, moderate to strong staining reaction.

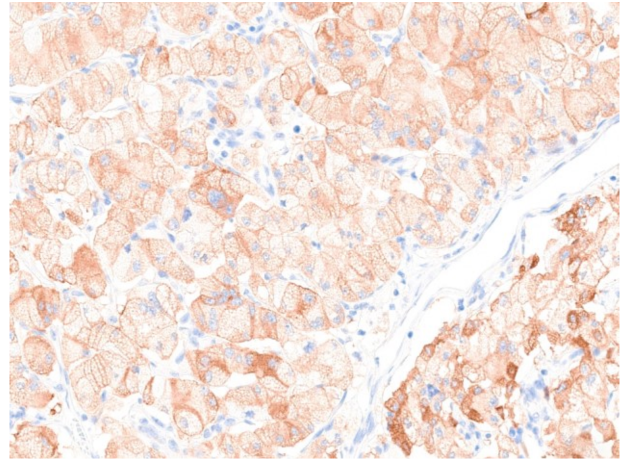


Fig. 3b (x200)
Staining reaction of SYP in the neuroendocrine tumour – same field as in Fig. 3a. Although the neoplastic cells display weak to moderate staining intensity, the protocol provides an overall too low analytical sensitivity – compare with Fig. 1a and 2b

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.

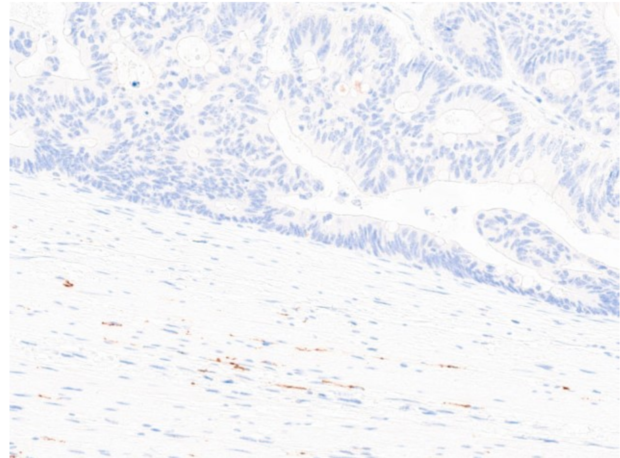


Fig. 4b (x200)
Staining reaction of SYP in the colon adenocarcinoma using same insufficient protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
The neoplastic cells are negative, but the peripheral nerves in the stromal compartment only display a faint to weak staining reaction.

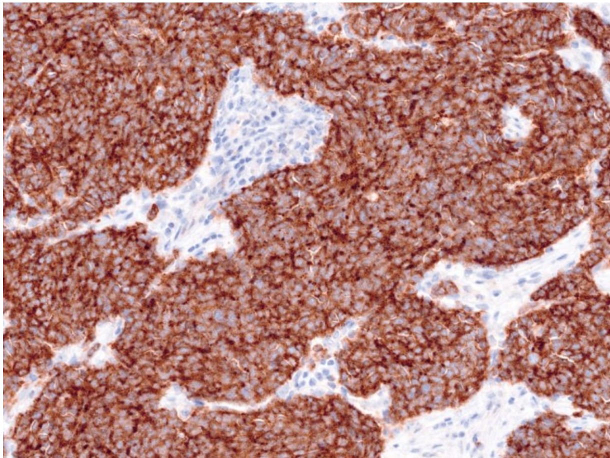


Fig. 5a (x200)
Optimal staining reaction for SYP in the SCLC using the rmAb clone BP6053 (RTU I10412E, Biolynx Biotechnology), with HIER in an alkaline buffer and a 3-layer detection system. All neoplastic cells show a strong and distinct staining reaction. Same pattern was observed with similar protocols using BS15 and MX038. No background staining is seen.
Same protocol used in Fig. 5b.

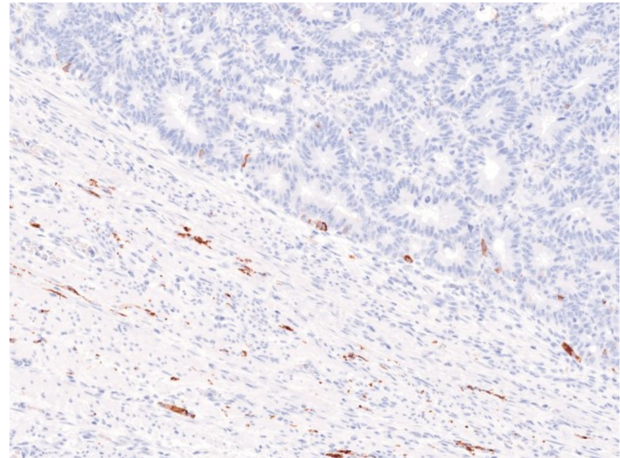


Fig. 5b (x200)
Optimal staining reaction of SYP in the colon adenocarcinoma using same protocol as in Fig. 5a. As expected, no staining of neoplastic cells is seen. The peripheral nerves in the stromal compartment show a strong and distinct cytoplasmic staining reaction.

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