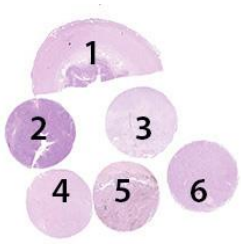
Assessment Run 59 2020
S100

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests for S100 among the NordiQC participants, used to identify melanocytic origin of cancer of unknown primary (CUP) origin and differentiate between schwannoma and leiomyoma.
Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of S100 antigen densities (see below).

Material
The slide to be stained for S100 comprised:



1. Appendix, 2. Tonsil, 3. Schwannoma, 4. Leiomyoma, 5-6. Malignant melanoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a S100 staining as optimal included:

- The vast majority of adipocytes must show an at least weak to moderate nuclear and cytoplasmic staining reaction.
- A strong, distinct nuclear and cytoplasmic staining reaction of virtually all Schwann cells of peripheral nerve fibres and ganglionic satellite cells in muscularis propria and submucosa in the appendix.
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of the vast majority of macrophages in all specimens.
- A strong, distinct nuclear and cytoplasmic staining reaction of virtually all neoplastic cells of the malignant melanomas (tissue cores no. 5-6) and the Schwannoma.
- No staining of other cells. The neoplastic cells in the leiomyoma, squamous epithelial cells in the tonsil, smooth muscle cells and columnar epithelial cells in the appendix should be negative.

In this assessment adipocytes were used as immunohistochemical critical assays performance controls (ICAPCs) in concordance with the guidelines and requirements for an optimal S100 staining reaction given by the International Ad Hoc Expert Committee[1]. In the previous NordiQC assessments follicular dendritic cells in tonsils were used as ICAPCs to monitor the level of analytical sensitivity, but the reaction in these cells are mainly seen for the pAb Z0311, Dako and caused by a reaction with alpha subunits in addition to beta (B) subunits. The main diagnostic application of S100 is demonstration of S100B expressed in e.g. melanomas and schwannomas which is the target/epitope for most routinely applied primary Abs. For this reason, the criteria for optimal performance was changed and aligned with the purpose of this assessment. In addition, the pAb Z0311 has been terminated from the vendor (Dako) and adjustment of expected test performance characteristics was required.

Participation

Number of laboratories registered for S100, run 59	368
Number of laboratories returning slides	299 (81%)

The number of laboratories returning slides has decreased in this run 59 compared to previous assessments due to the COVID-19 pandemic. All slides returned after the assessment will be assessed, and receive advice if the result is insufficient, but will not be included in this report.

Results
299 laboratories participated in this assessment. 244 (82%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Insufficient HIER (too low temperature and/or too short efficient heating time)
- Proteolytic pre-treatment or omission of epitope retrieval
- Unexplained technical issues

Performance history
This was the sixth NordiQC assessment of S100. Despite a change in the assessment criteria, the overall pass rate was the same as the previous run for S100 (see Table 2).

Table 2. **Proportion of sufficient results for S100 in the sixth NordiQC runs performed**

	Run 7 2003	Run 20 2007	Run 34 2012	Run 45 2015	Run 50 2017	Run 59 2020
Participants, n=	63	106	200	251	298	299
Sufficient results	71%	75%	64%	68%	82%	82%

Conclusion
pAbs, both as concentrated and Ready-To-Use (RTU) formats, were most successful for the immunohistochemical demonstration of S100. Laboratory developed assays based on pAb Z0311 provided the highest proportion of optimal results. Irrespective of the primary Ab applied, efficient HIER and use of appropriate titre and incubation time tailored to the choice of IHC system were the most important prerequisites for a sufficient staining result. Omission of HIER and/or use of proteolytic pre-treatment provided inferior performance.
Appendix is recommended as positive and negative tissue control: The Schwann cells of peripheral nerves, macrophages/dendritic cells must show a moderate to strong distinct nuclear and cytoplasmic staining reaction. The vast majority of adipocytes must show an at least weak but distinct nuclear and cytoplasmic staining reaction. No staining reaction must be seen in other cell types including smooth muscle cells in lamina muscularis propria and columnar epithelial cells of the appendix.
In addition, tonsil can also be included as positive tissue control when pAb Z0311 is used. Virtually all interfollicular dendritic cells and Langerhans cells of the squamous epithelium must show a moderate to strong nuclear and cytoplasmic staining reaction, whereas the follicular dendritic cells (meshwork) must display an at least weak to moderate but distinct nuclear and cytoplasmic staining reaction.

Table 1. **Antibodies and assessment marks for S100, run 59**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **4C4.9**	1 1 2 1 1 2 2 1	Thermoscientific Immunologic Cell Marque Diagnostic BioSystems DCS BioCare Medical Zytomed Systems Zeta Corporation	2	5	2	2	63%	18%
mAb clone **15E2E2**	2	Biocare	-	2	-	-	-	-
mAb clone **15E2E2+4C4.9**	1	Biocare	1	-	-	-	-	-
mAb **IHC-100-1**	1	GenomeMe	-	1	-	-	-	-
rmAb **EP32**	1	Leica Biosystems	-	1	-	-	-	-
pAb **Z0311**[5]	100	Agilent/Dako	55	27	15	3	82%	55%
pAb **NCL-L-S100p**	8	Leica/Novocastra	1	4	2	1	62%	13%
pAb **E031**	1	LINARIS	-	1	-	-	-	-
pAb **254-2F52B2**	1	Biosite	-	1	-	-	-	-
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **4C4.9** **790-2914 (VRPS)**[3]	4	Roche/Ventana	-	4	-	-	-	-
mAb clone **4C4.9** **790-2914 (LMPS)**[4]	33	Roche/Ventana	9	15	8	1	73%	27%
mAb clone **4C4.9** **330M-17/18/10**	1	Cell Marque	1	-	-	-	-	-
mAb clone **4C4.9** **MAD-001221**	2	Vitro SA	-	-	2	-	-	-
rmAb **EP32** **AN713-5M/10M**	1	BioGenex	1	-	-	-	-	-
rmAb **EP32** **8442-C010**	2	Sakura Finetek	1	-	1	-	-	-
pAb **IS/IR504 (VRPS)**[3]	6	Agilent/Dako	4	2	-	-	100%	67%
pAb **IS/IR504 (LMPS)**[4]	19	Agilent/Dako	14	4	1	-	95%	74%
pAb **GA504 (VRPS)**[3]	29	Agilent/Dako	28	1	-	-	100%	97%
pAb **GA504 (LMPS)**[4]	17	Agilent/Dako	13	3	1	-	94%	77%
pAb **760-2523 (VRPS)**[3]	11	Roche/Ventana	3	7	1	-	91%	27%
pAb **760-2523 (LMPS)**[4]	32	Roche/Ventana	8	15	9	-	72%	25%
pAb **PA0900 (VRPS)**[3]	3	Leica/Novocastra	-	-	3	-	-	-
pAb **PA0900 (LMPS)**[4]	10	Leica/Novocastra	1	6	3	-	70%	10%
Unknown	3	Unknown	-	3	-	-	-	-
Total	299		142	102	48	7	-	
Proportion			48%	34%	16%	2%	82%	

1) Proportion of sufficient stains (optimal or good). (≥5 assessed protocols)
2) Proportion of Optimal Results (≥5 assessed protocols).
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 (≥5 assessed protocols).
5) Ab terminated by vendor.

Detailed analysis of S100, Run 59

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

pAb **Z0311**: Protocols with optimal results were typically based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1, Ventana) (39/53)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) or TRS pH 9 (11/17), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (4/8), TRS pH 6 (Dako) (1/2) as retrieval buffer. The pAb was typically diluted in the range of 1:1,000-4,000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 54 of 58 (93%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb **4C4.9:** Two protocols provided an optimal result, both based on HIER using CCC1 (Ventana) (2/4) as retrieval buffer. The mAb was diluted in the range of 1:400-600 depending on the total sensitivity of the protocol employed. Using these protocol settings, 3 of 4 (75%) laboratories produced a sufficient staining result.

pAb **NCL-L-S100p**: One protocol with an optimal result was based on HIER for 15 min. using Tris-EDTA/EGTA pH9 (1/1) as retrieval buffer. The pAb was diluted 1:600 using a 2-layer detection kit (GTVision) and was performed on a Gene Tech Genestainer.

Table 3. **Proportion of optimal results for S100 for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica 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
pAb **Z311**	5/8** (63%)	1/2	6/9 (67%)	-	39/53 (65%)	0/2	4/8 (50%)	0/4
mAb **4C4.9**	-	-	-	-	2/4	-	-	0/1

* 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 **4C4.9**, product no. **790-2914**, Roche/Ventana, Ventana Benchmark:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 8-20 min. at 90-100°C), 8-24 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection systems. Using these protocol settings, 24 of 30 (80%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 100% (4/4) and 0% (0/4) of the laboratories produced an optimal staining result (see Table 4).

pAb, product no. **IS/IR504**, Agilent/Dako, Autostainer:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 96-97°C), 10-30 min. incubation of the primary Ab and EnVision Flex (K8000) as detection system. Using these protocol settings, 11 of 11 (100%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 100% (6/6) and 67% (4/6) of the laboratories produced an optimal staining result (see Table 4).

pAb, product no. **GA504**, Agilent/Dako, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH 9 (GV804) (efficient heating time 30 min. at 97°C) and 15-20 min. incubation of the primary Ab and EnVision FLEX (GV800) as detection system. Using these protocol settings, 40 of 40 (100%) laboratories produced a sufficient staining result. Applying VRPS, the proportion of sufficient results was 100% (29/29) and 97% (28/29) of the laboratories produced an optimal staining result (see Table 4).

pAb, product no. **760-2523**, Roche/Ventana, Ventana Benchmark:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 8-44 min. at 95-100°C), 12-32 min. incubation of the primary Ab and UltraView with or without amplification (760-500/760-080) or OptiView (760-700) as detection systems. Using these protocol settings, 25 of 30 (83%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 91% (10/11) and 27% (3/11) of the laboratories produced an optimal staining result (see Table 4).

pAb, product no. **PA0900**, Leica/Novocastra, Bond:
One protocol with an optimal result was based on HIER using BERS2 (efficient heating time 30 min. at 100°C), 30 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 1 laboratory produced a sufficient result. Applying VRPS, the proportion of sufficient results was 0% (0/3).

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly 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 4. **Proportion of sufficient and optimal results for S100 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS pAb **IS/IR504**	100% (6/6)	67% (4/6)	100% (9/9)	78% (7/9)
Dako Omnis pAb **GA504**	100% (29/29)	97% (28/29)	100% (15/15)	80% (12/15)
Leica BOND MAX/III pAb **PA0900**	0% (0/3)	0% (0/3)	70% (7/10)	10% (1/10)
VMS Ultra/XT pAb **760-2523**	91% (10/11)	27% (3/11)	72% (23/32)	25% (8/32)
VMS Ultra/XT mAb 4C4.9 **790-2914**	100% (4/4)	0% (0/4)	73% (24/33)	27% (9/33)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Comments

In this assessment and in concordance with the previous NordiQC S100 assessments, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 66% (36 of 55) of the insufficient results. The majority of participating laboratories were able to demonstrate S100 in Schwann cells of peripheral nerves, whereas the demonstration of S100 in adipocytes (all Abs) and follicular dendritic cells (pAbs based on Z0311) in the tonsil was more challenging and only seen with appropriate protocol settings. It was observed, that lack of reaction of adipocytes and the follicular dendritic cells resulted in weaker or too weak staining intensity of the Schwannoma and both malignant melanomas – especially in the melanoma tissue tissue core no. 6 (Figs. 4a+b).
For the remaining insufficient results, the staining patterns were characterized by a poor signal-to-noise ratio, false positive staining reaction and/or impaired morphology due to proteolytic pre-treatment compromising the interpretation (Figs. 8a and 8b).

42% (126 of 299) of the laboratories used concentrated Ab formats within laboratory developed (LD) assays for S100. The pAb Z0311 was the most widely used Ab and could be used to obtain optimal staining results as shown in Tables 1 and 3. Used within a LD assay, the pAb Z0311 gave an overall pass rate of 82% (82 of 100) and 55% (55 of 100) optimal.
HIER, preferable in an alkaline buffer, in combination with a careful calibration of the primary Ab seem to be the most critical parameters for sufficient and optimal results (see Table 5). Omission of HIER or performing proteolytic pre-treatment was less successful. Using HIER and a titer of the primary Ab Z0311 in the range of 1:1,000-1:4,000, 93% (54 of 58) of the protocols provided a sufficient result of which 79% (46 of 58) was optimal. In comparison, using the same primary Ab and titer range but omitting HIER or using proteolytic pre-treatment, 43% (3 of 7) of protocols gave a sufficient result of which none were assessed as optimal.
Compiling the data from the last three NordiQC runs for S100, the observations mentioned above were confirmed as shown in Table 5. In general, the 4 Abs included in Table 5 all have a higher pass rate when using HIER (total 81%) compared to proteolytic pretreatment (total 47%) and no pretreatment (total 50%).

Table 5. **Pass rates for S100 antibody combined with epitope retrieval methods in the last three NordiQC runs**

Pass rate for compiled data from run 45, 50 & 59										
	Total		**HIER**		**Proteolysis**		**HIER + proteolysis**		**No pretreatment**	
	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient	Protocols	Sufficient
mAb 4C4.9	137	80 (58%)	110	71 (65%)	4	0	2	1	21	8 (38%)
pAb NCL-L-S100p	30	18 (60%)	21	14 (67%)	6	2 (33%)	0	0	3	2
pAb Z0311	494	417 (84%)	444	386 (87%)	26	15 (58%)	3	2	21	14 (67%)
pAb 760-2523	97	68 (70%)	82	62 (76%)	2	1	0	0	13	5 (39%)
Total	758	583 (77%)	657	533 (81%)	38	18 (47%)	5	3	58	29 (50%)

In this run, there was no significant difference in the overall performance related to the choice of the detection system. Using optimal protocol settings for the pAb Z0311 as concentrate, the pass rates for 2- and 3-step multimer/polymer detection systems were 93% (28 of 30) and 86% (31 of 36), respectively, of which 70% (21 of 30) and 69% (25 of 36) were optimal.
Precise calibration of the primary Ab was a central parameter for optimal performance. If HIER was used in combination with a titer below 1:1,000, a poor signal-to-noise ratio frequently was seen hampering interpretation. If a titer in the range of 1:5,000-10,000 was used, typically a general too weak staining reaction was seen.

Eight laboratories used the pAb NCL-L-S100p within a LD assay and 62% (5 of 8) produced a sufficient result of which 13% (1 of 8) were assessed as optimal. The single protocol with an optimal result was stained on a Gene Tech Genestainer based on HIER (Tris-EDTA/EGTA pH 9), diluted 1:600 (50 min. incubation) and using GTVision as detection system. The pAb was mainly applied on the Leica BOND platforms, BOND-III/MAX (4 of 8 protocols), none provided an optimal result.

In general, the performance for S100 within LD assays was identical on the 4 main IHC platforms using optimized protocol settings (see Table 3). For unexplained reasons, a few protocols based on the pAb Z0311 gave an unexpected and aberrant nuclear staining reaction in scattered cells in the leiomyoma, when applied on the Ventana Benchmark platform. This was observed in both UltraView and OptiView based protocols This result was accepted if the protocols otherwise provided the expected results. In cases with extended staining reaction in the neoplastic cells, protocols were downgraded - see Figs. 5a and 5b). No explanation of the aberrant reaction pattern could be identified from the protocols submitted.

58% (173 of 299) of the laboratories used Ready-To-Use (RTU) systems for the demonstration of S100, which is an increase compared to 46% in run 50. Especially the RTU systems GA504 for Dako Omnis, 760-2523 for Ventana BenchMark and PA0900 for Leica Bond has gained popularity and is now almost used by twice as many participants compared to last run.

The Dako RTU products IS/IR504 and GA504 based on a pAb with intended use for Dako Autostainer and Omnis, respectively was in total used by 71 participants (24%). As seen in Tables 1 and 4 the overall pass rate and proportion of optimal results was very high, both for VRPS and LMPS. The Omnis RTU system performed as "Plug-and-Play" in compliance with vendor recommended protocol settings was most successful with a pass rate of 100% and 97% being optimal. LMPS, exchanging DAB with Magenta, was found to be less successful, especially caused by difficulties in visualizing the nuclear expression of S100 in the melanoma, tissue core no. 6. (see Fig. 6b). Internal NordiQC experiences have revealed similar challenges for other nuclear markers exchanging DAB with Magenta as chromogen.

The Ventana RTU format 760-2523 based on a pAb for the BenchMark platforms was used by 43 participants (14%) and, as seen in Tables 1 and 4, provided a relatively high pass rate (77%), especially when applying VRPS. However, a relatively low proportion of optimal results was observed (26%). In general, a too weak staining reaction and/or reduced number of cells demonstrated was typically seen.

The RTU system 790-2914 (Ventana) based on the mAb 4C4.9, used by 37 particpants (12%), gave a higher proportion of sufficient results compared with the latest run 50 (76% vs. 56%), but only 24% optimal (9/37) (see Table 1). The nine optimal protocols were based on laboratory modified protocol

settings typically prolonging the incubation time of primary Ab and/or prolonging HIER compared to the vendor recommendations. The typical pattern of insufficient results was either a too weak staining intensity of cellular structures expected to be demonstrated or a false positive staining reaction of e.g. smooth muscle cells of lamina muscularis propria in the appendix (see Fig. 7a). If the RTU format was used without HIER, irrespective of incubation time of the primary Ab and detection system (UltraView or OptiView), a pass rate of 38% (8 of 21) was seen.

13 participants used the Leica RTU PA0900 system based on a pAb. Three of the participants used the vendor recommended protocol settings based on enzymatic pre-treatment, none received a sufficient result. All laboratory modified protocol settings, adjusting pre-treatment and using HIER instead of enzymatic digestion, and/or prolonged incubation time in primary ab, provided a sufficient staining result.

This was the sixth assessment of S100 in NordiQC (see Table 2). A pass rate of 82% was obtained, which is equivalent with the latest run 50, 2017. The proportion of protocols that provided an optimal result was however significantly increased from 23% in the latest run to 48% in this run. The modified assessment criteria using adipocytes as ICAPCs on the expense of follicular dendritic cells did not affect the overall pass rate, but might have contributed to the increased proportion of optimal results. In this run, only very few protocols demonstrated S100 in the follicular dendritic cells and as seen in the latest run, especially RTU systems were challenged to accomplish this staining pattern. Most importantly the change of ICAPCs did not affect the analytical sensitivity for the melanomas and schwannoma included in the circulated material.

In general, polyclonal antibodies gave a superior performance compared to monoclonal antibodies. 237 laboratories submitted protocols based on polyclonal antibodies and 84% (198 of 237) received a sufficient result, 54% optimal, whereas monoclonal antibodies were applied in 62 protocols, 74% with a sufficient mark (46 of 62), only 24% optimal. The pAb Z0311 has been terminated from Dako and will gradually be replaced by other products. pAbs for S100 are available as RTU systems from e.g. Dako and Ventana, but there is still a need to identify alternatives to the "Gold standard for S100", Z0311 especially for laboratories wanting to use concentrated formats. One of the recently launched antibodies is rmAb clone EP32 reacting with S100B, being available both as concentrated format and within RTU systems as shown in Table 1. Data and studies comparing the analytical concordance of Z311 and EP32 applied by optimal protocol settings are warranted for an exchange of the "classical S100 Ab" to e.g. EP32.

1. Guidelines and requirements for an optimal S100 staining reaction given by the International Ad Hoc Expert Committee (Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18.).

Controls
Appendix is recommended as primary positive and negative tissue control for S100. In the appendix virtually all adipocytes and Schwann cells of peripheral nerves, must show an as strong as possible nuclear and cytoplasmic staining reaction without any staining reaction of the smooth muscle or epithelial cells. As supplement when using pAb Z0311 tonsil can be used. In the tonsil, interfollicular dendritic cells and Langerhans cells of the squamous epithelium, must display a moderate to strong staining intensity whereas the follicular dendritic cell meshwork of the germinal centres should show an at least weak to moderate nuclear and cytoplasmic staining reaction.

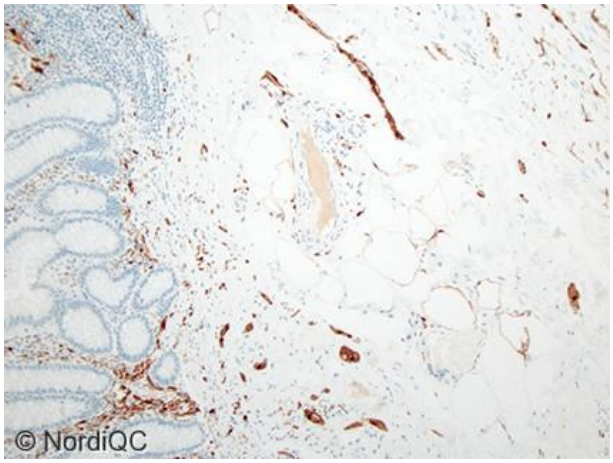


Fig. 1a (x100)
Optimal staining reaction for S100 of appendix using the pAb Z0311 as concentrate, HIER in an alkaline buffer (TRS pH 9) and a polymer-based detection system (FLEX, Agilent/Dako) - same protocol used in Figs. 2a - 5a. The adipocytes and Schwann cells of the peripheral nerves show a moderate to strong staining reaction without any background staining - compare with Fig. 1b.

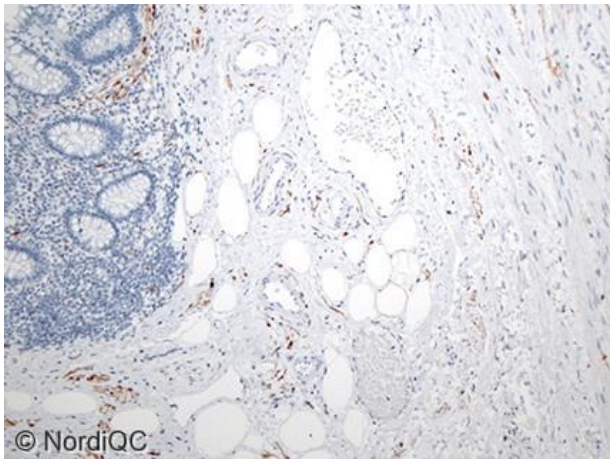


Fig. 1b (x100)
Insufficient staining reaction for S100 of the appendix using the pAb Z0311 as concentrate (too diluted), HIER in an alkaline buffer (too short time) and a polymer-based detection system (FLEX, Agilent/Dako) - same protocol used in Figs. 2b - 4b. The intensity of the staining reaction is significantly reduced, and the majority of adipocytes are negative or only show a weak staining reaction - compare with Fig. 1a.

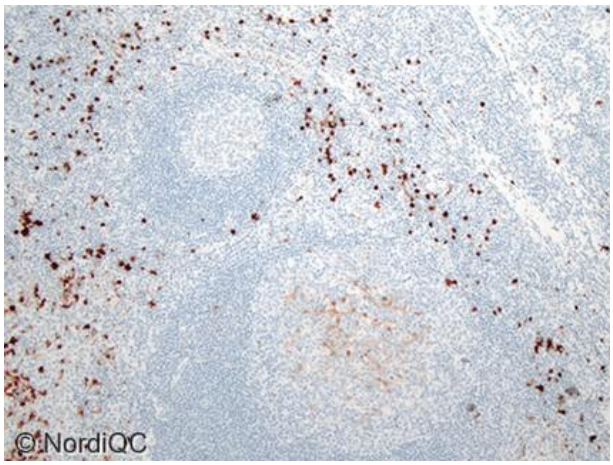


Fig. 2a (x100)
Optimal staining reaction for S100 of the tonsil using the same protocol as in Fig. 1a. The majority of the interfollicular macrophages, dendritic cells and Langerhans cells in the squamous epithelium display a strong staining reaction, while the follicular dendritic cells in germinal centres show a weak to moderate but distinct nuclear and cytoplasmic staining reaction - compare with Fig. 2b.

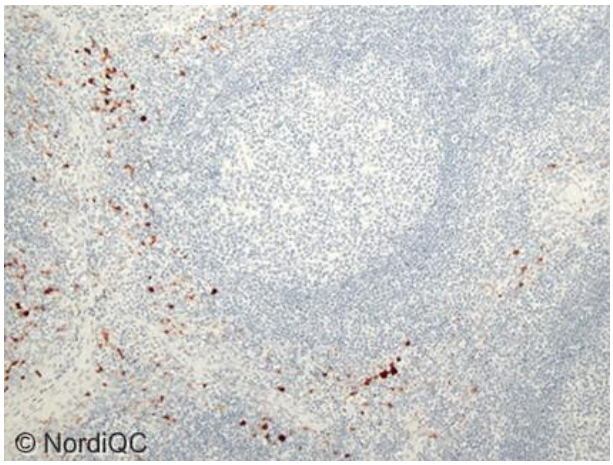


Fig. 2b (x100)
Insufficient staining reaction for S100 of the tonsil using same protocol as in Fig. 1b - same field as in Fig. 2a. The proportion of positive cells and the intensity of the staining reaction being significantly reduced.

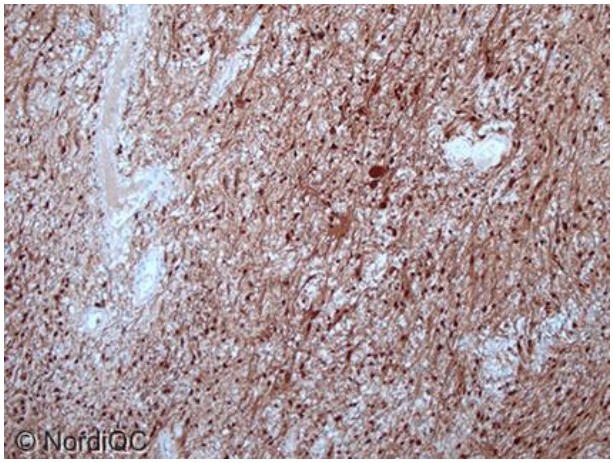


Fig. 3a (x100)
Optimal staining reaction for S100 of the schwannoma using the same protocol as in Figs. 1a and 2a. All neoplastic cells display a strong nuclear and cytoplasmic staining reaction.

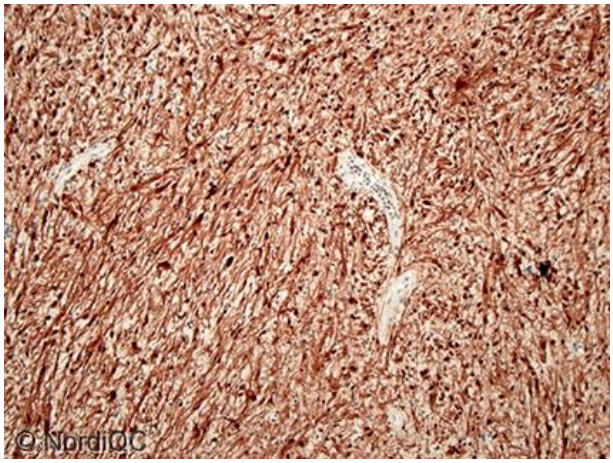


Fig. 3b (x100)
Staining reaction for S100 of the Schwannoma using the same insufficient protocol as Figs. 1b and 2b. The staining intensity is almost identical compared to Fig. 3a and emphasizes the importance to use appropriate tissue controls with relevant and critical expression levels. A schwannoma with high level expression cannot reliably monitor a correctly calibrated and/or performed test for S100 – see also Fig. 4b.

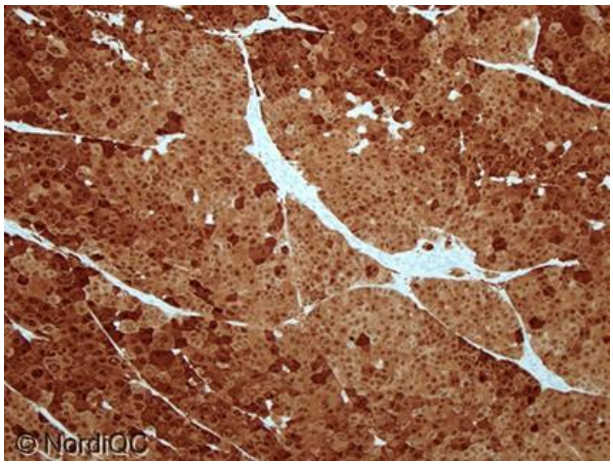


Fig. 4a (x100)
Optimal staining reaction for S100 of the malignant melanoma, tissue core no. 6, using same protocol as in Figs. 1a – 3b. All neoplastic cells show a strong nuclear and cytoplasmic staining reaction – compare with Fig. 4b.

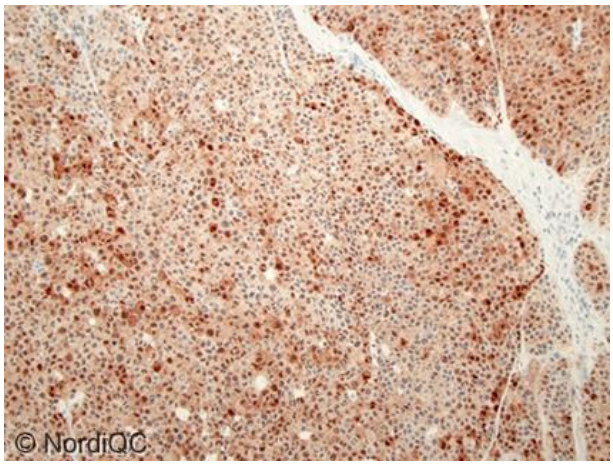


Fig. 4b (x100)
Insufficient staining reaction for S100 of the malignant melanoma, tissue core no. 6, using same protocol as in Figs. 1b - 3b – same field as in Fig. 4a.
Most of the neoplastic cells only show a weak to moderate cytoplasmic staining reaction, whereas a large number of the nuclei are false negative, and the interpretation compromised due to the change in reaction pattern for S100

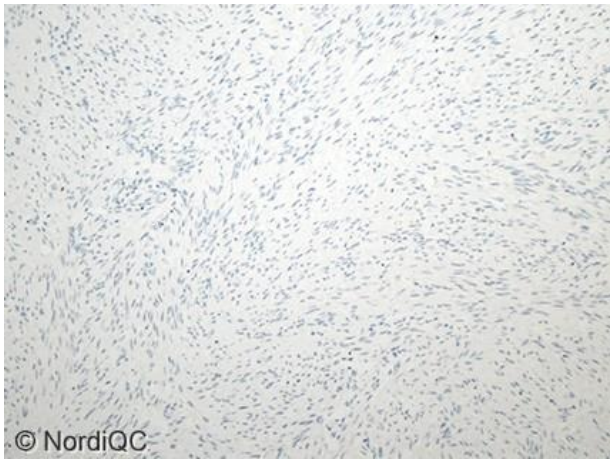


Fig. 5a (x100)
Optimal staining reaction of the leiomyoma using same protocol as Figs. 1a – 4a. None of the neoplastic cells display any staining reaction.

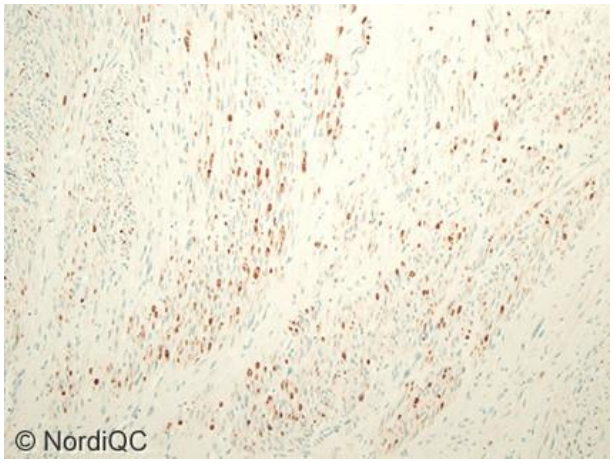


Fig. 5b (x100)
Insufficient staining reaction for S100 of the leiomyoma using the pAb Z0311 as concentrate, (HIER in an alkaline buffer and a polymer-based detection system (UltraView, Ventana). The majority of neoplastic cells show a false positive nuclear staining reaction, with faint cytoplasmic staining as well. Compare with Fig. 5a. A focal reaction was accepted, providing otherwise optimal staining result.

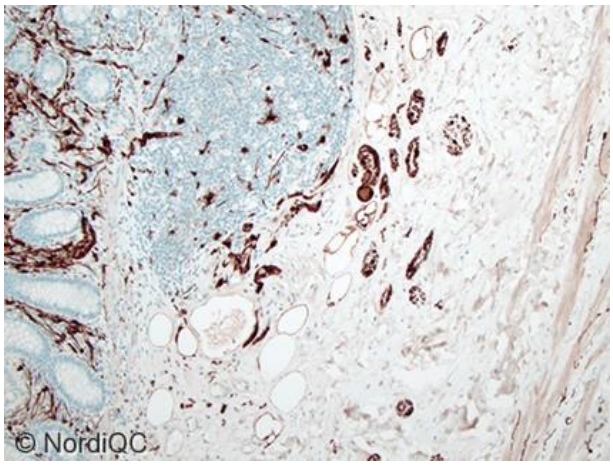


Fig. 6a (x100)
Insufficient staining reaction for S100 of the appendix using the mAb clone 4C4.9 as RTU format (790-2914, Roche/Ventana) with HIER in CC1 (36 min.) and UltraView (Roche/Ventana) as detection system and performed on the Ventana Benchmark Ultra. An aberrant cytoplasmic staining reaction in especially the smooth muscle cells of the vascular structures and a diffuse background reaction complicates the interpretation.
Compare with Fig. 1a.

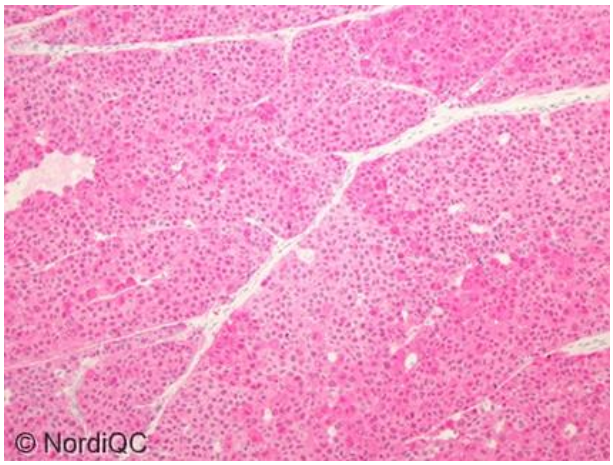


Fig. 6b (x100)
Sufficient staining reaction for S100 of the malignant melanoma, tissue core no. 6, using the pAb RTU format GA504 (Agilent/Dako) vendor recommended protocol but applying EnVision Flex HRP Magenta instead of DAB. The nuclei only display a weak positive staining reaction compared to the level and pattern expected (see Fig. 4a. All other components were demonstrated as expected including the adipocytes in the appendix.

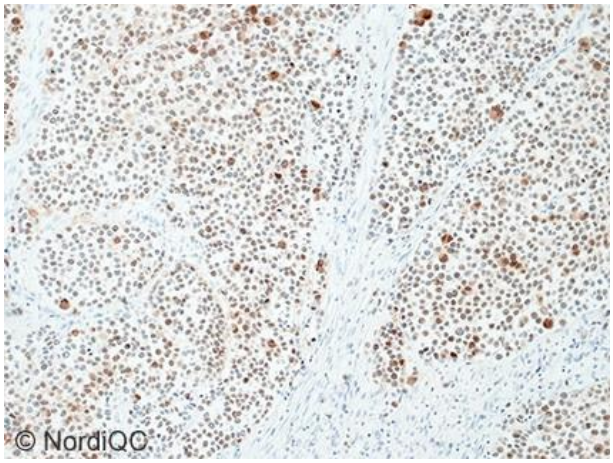


Fig. 7a (x100)
Insufficient staining reaction for S100 of the melanoma, tissue core no. 6, using the pAb RTU format, PA09000 (Leica). Most of the neoplastic cells show a weak to moderate nuclear staining reaction but the cytoplasmic compartment in most cells is digested by enzymatic pre-treatment and only few cells also display a weak cytoplasmic staining reaction.
This staining pattern was typically seen when proteolysis was applied as pre-treatment hampering the morphology in fragile tissues. Same protocol as in Fig. 7b.

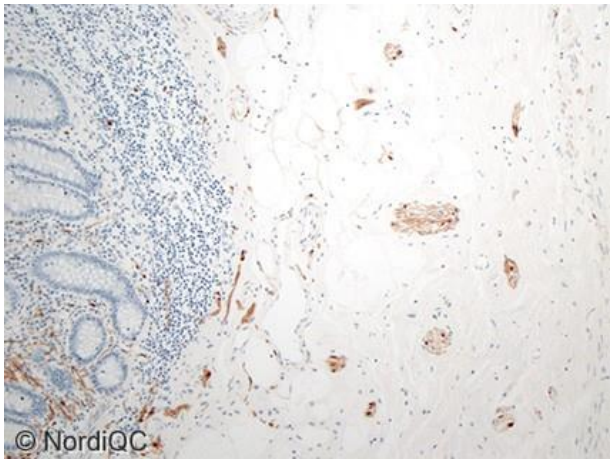


Fig. 7b (x100)
Insufficient staining reaction for S100 of the appendix using same protocol as Fig. 7a. The adipocytes are almost completely negative, and the number and intensity of positive Schwann cells of the peripheral nerves are strongly reduced compared to Fig. 1a.

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