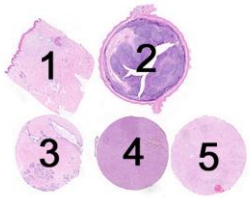
Assessment Run 34 2012

S-100 PROTEIN (S100)

Material
The slide to be stained for S100 comprised:

1. Skin, 2. Appendix , 3. Breast hyperplasia, 4. Malignant melanoma, 5. Schwannoma



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an S100 staining as optimal included:

- A strong, distinct nuclear and cytoplasmic staining reaction of the normal melanocytes, the Langerhans cells and (when present) the myoepithelial cells of the sweat glands in the skin. No staining should be seen in the squamous epithelial cells.
- A strong, distinct nuclear and cytoplasmic staining reaction of the macrophages in lamina propria, the Schwann cells (of the peripheral nerve fibres) and the ganglionic satellite cells in the muscularis propria and submucosa in the appendix. The epithelial cells and muscle cells should be negative.
- An at least weak but distinct nuclear and cytoplasmic staining reaction of the follicular dendritic cells in the germinal centres of the Peyer's plaques in the appendix
- A moderate to strong, distinct nuclear and cytoplasmic staining reaction of the myoepithelial cells in the breast, and no more than a focal reaction in the epithelial cells.
- A strong, distinct nuclear and cytoplasmic staining reaction of the majority of the neoplastic cells of the malignant melanoma and the schwannoma.
- A strong, distinct nuclear and cytoplasmic staining reaction of the fat cells and macrophages in all specimens.

200 laboratories participated in this assessment. 64 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for S100, run 34**

Concentrated Abs:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **4C4.9**	1 1 1	Master Diagnostica Thermo/NeoMarkers Zytomed	0	2	0	1	-	-
mAb clone **12E2E2**	1	Biogenex	0	0	1	0	-	-
mAb clone cocktail **12E2E2+4C4.9**	1	Biocare	0	1	0	0	-	-
rmAb clone **EP32**	2	Epitomics	0	2	0	0	-	-
pAb **Z0311**	131	Dako	36	46	35	14	63 %	80 %
pAb **NCL-S100p**	5	Leica/Novocastra	0	3	2	0	60 %	-
pAb **RB-9018-p**	3	Thermo/NeoMarkers	0	3	0	0	-	-
pAb **E031**	1	Linaris	0	0	0	1	-	-
Ready-To-Use Abs:								
mAb clone **4C4.9 330M-17**	1	Cell Marque	0	0	0	1	-	-
mAb clone **4C4.9 MON-RTU1191**	1	Monosan	0	0	0	1	-	-
mAb clone **4C4.9 790-2914**	12	Ventana	0	5	4	3	42%	-
mAb clone cocktail **12E2E2+4C4.9 PM089**	1	Biocare	0	0	0	1	-	-

pAb **IR/IS504**	24	Dako	0	23	1	0	96 %	-
pAb **760-2523**	10	Ventana/Cell Marque	0	2	7	1	20 %	-
pAb clone **PA0900**	3	Leica	0	3	0	0	-	-
pAb	1	Unknown	0	1	0	0	-	-
Total	200		36	91	50	23	-	
Proportion			18 %	46 %	25 %	11 %	64 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs:
pAb **Z0311**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Cell Conditioning 1 (CC1, BenchMark, Ventana) (12/40)*, Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9 (3-in-1), Dako)(9/18/)*, TRS pH 9 (Dako) (5/11)*, Bond Epitope Retrieval Solution 2 (BERS 2, Bond, Leica) (4/9)*, Bond Epitope Retrieval Solution 1 (BERS 1, Bond, Leica) (1/1)*, Tris-EDTA/EGTA pH 9 (3/6)* or Citrate pH 6 (2/6)* as the retrieval buffer.
The mAb was typically diluted in the range of 1:750-1:10.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 67 out of 84 (80 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Less successful primary antibody
- Proteolytic pre-treatment
- Omission of epitope retrieval

In this assessment and in concordance with the observations in the previous assessment of S100 (run 20, 2007), the prevalent feature of an insufficient staining was a too weak or completely false negative reaction in the cells supposed to be demonstrated. A too weak or false negative staining was seen in 88 % of the insufficient results (64 out of 73). Virtually all the participating laboratories were able to demonstrate S100 in the peripheral macrophages, the Schwann cells and the neoplastic cells of the schwannoma, whereas the demonstration of S100 in the myoepithelial cells, the Langerhans cells and in particular the follicular dendritic cells of the germinal centres was much more challenging and was only seen with appropriate protocol settings, e.g., a correct titre of the pAb Z0311, Dako and the use of HIER.
Omission of epitope retrieval gave a significantly reduced sensitivity: In 18 out of 25 protocols without epitope retrieval but otherwise optimal protocol settings an insufficient result was obtained and none were assessed as optimal.
Virtually the same insufficient results were seen when proteolytic pre-treatment was used: 15 out of 23 protocols gave an insufficient result and none were optimal. The use of proteolysis both caused a reduced sensitivity and an impaired morphology as in several cases the cytoplasmic compartment of many cells e.g., the neoplastic cells of the melanoma and the epithelial cells of the breast was totally extracted due to an excessive digestion.

In this run only the pAb Z0311, Dako applied with HIER and diluted in the range of 1:750 - 10.000 of the concentrated format could be used to obtain to optimal staining reaction for S100 in all the tissues. Using these protocol settings, the overall pass rate was 80 %. For the Ready-To-Use (RTU) product of the same pAb IR/IS504, Dako, a pass rate of 100 % was seen if same protocol settings were applied. However, none were assessed as optimal, primarily due to a slightly reduced sensitivity compared to the staining obtained by the concentrated format.

Appendix was found to the most reliable and informative control for S100. In the optimal protocols an at least weak to moderate but distinct nuclear and cytopplasmic staining reaction in the follicular dendritic cells of the Peyer's plaques were seen indicating that these cells might be used as critical staining quality indicator for S100. The Schwann cells must be stained as strongly as possible, but still the smooth muscle cells and the epithelial cells must be negative.
In this context it might be superior to use tonsil as positive control for S100, as the number of lymphoid follicles in tonsils is more constant compared to appendix. Prompt and adequate fixation in formalin is crucial to stabilize and preserve S100 in cells and structures with a relatively low level of the S100 antigen as e.g. the follicular dendritic cells.

This was the 3rd assessment of S100 in NordiQC (Table 2). A decrease in the pass rate was seen from both run 7, 2003, and run 20, 2007.

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

	Run 7 2003	Run 20 2007	Run 34 2012
Participants, n=	63	106	200
Sufficient results	71 %	75 %	64 %

The lower pass rate may be due to several factors: New tissue material circulated and many new participants. The laboratories participating for the first time obtained a significantly lower pass rate compared to the laboratories also participating in the previous run 22, 2007: For the laboratories participating for the first time the pass rate was 57 % (59 out of 102), whereas the pass rate was 71 % (68 out of 106 laboratories) for the laboratories participating in both runs.

Conclusion

In this assessment the pAb Z0311 was the only antibody giving an optimal staining for S100. HIER and an appropriate titre of the primary antibody were mandatory for an optimal performance. Omission of epitope retrieval or proteolytic pre-treatment gave a significantly inferior result.
Appendix (or tonsil) is recommendable as positive control provided that the follicular dendritic cells show an at least weak to moderate distinct nuclear and cytoplasmic staining reaction. A strong staining reaction must be seen in the Scwann cells. No or only a minimal staining reaction should be seen in the smooth muscle cells

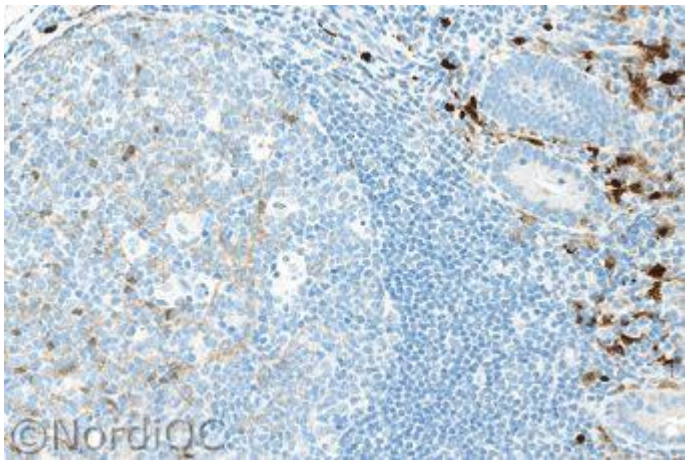


Fig 1a
Optimal staining for S100 of the appendix using the pAb Z0311 optimally calibrated and with HIER in an alkaline buffer. The peripheral nerves and macrophages in lamina propria show a strong cytoplasmic and nuclear staining reaction and a weak to moderate staining reaction is seen in the follicular dendritic cells in the germinal centre. No background staining is seen. Also compare with Figs. 2a – 4a, same protocol.

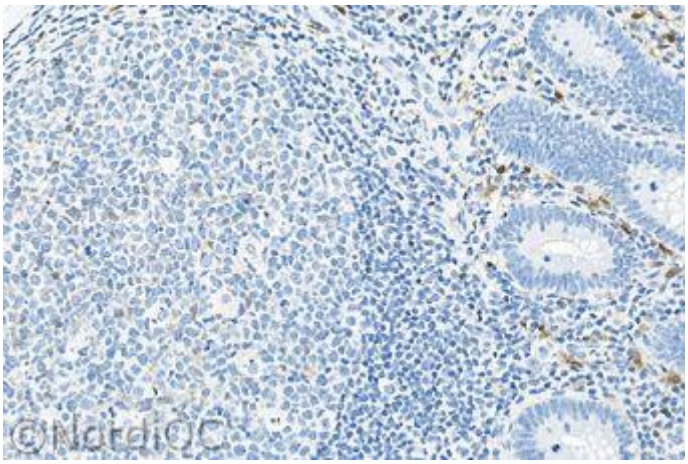


Fig 1b
Insufficient staining for S100 of the appendix using the pAb Z0311 by a protocol giving a too low sensitivity – too low concentration of the primary Ab., same field as in Fig. 1a. Virtually no staining reaction is seen in the follicular dendritic cells in the germinal centre and the staining reaction in the macrophages and peripheral nerves is significantly reduced – also compare with Figs. 2b - 4b, same protocol.

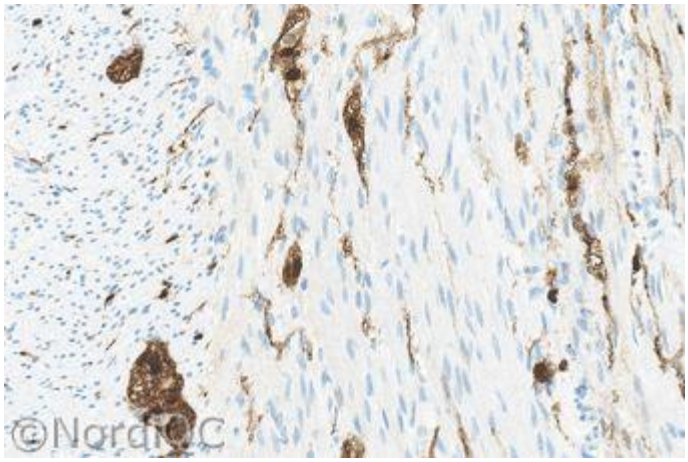


Fig 2a
Optimal staining for S100 of the appendix using the same protocol as in Fig. 1a. The Schwann cells and the satellite cells of the peripheral nerves show a moderate to strong staining reaction.
No staining is seen in the smooth muscle cells.

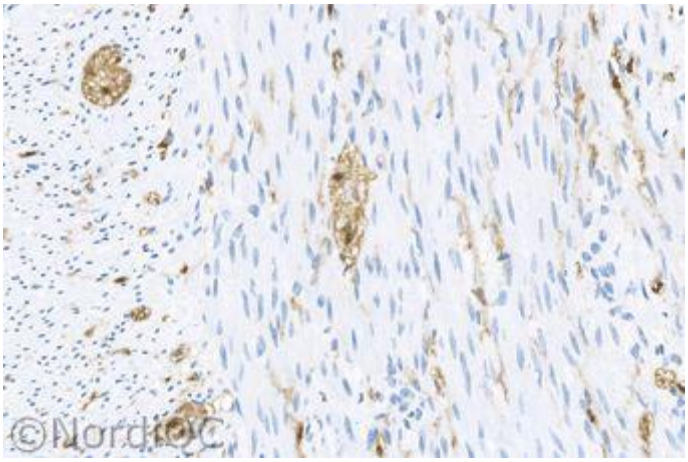


Fig 2b
Staining for S100 of the appendix using same protocol as in Fig. 1b., same field as in Fig. 2a. The proportion and intensity of the positive cells is reduced. However still a weak to moderate staining reaction is seen, indicating that peripheral nerves can not reliably be used as positive control for S100 – also compare with Figs. 3b - 4b same protocol.

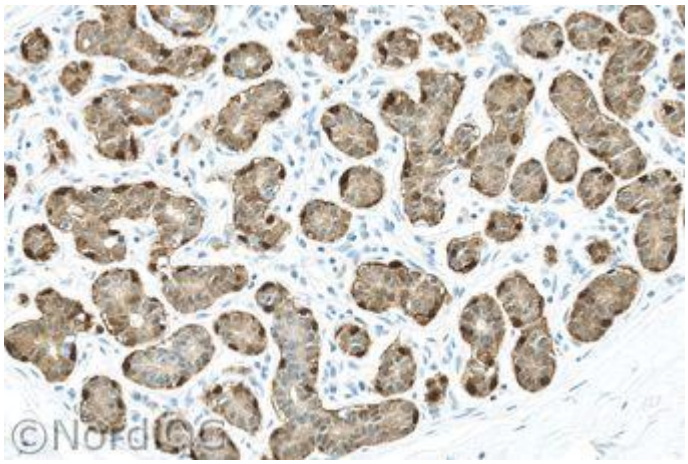


Fig 3a
Optimal staining for S100 of the breast hyperplasia using same protocol as in Figs. 1a & 2a. The myoepithelial cells show a moderate to strong cytoplasmic and nuclear staining reaction. No background reaction is seen.

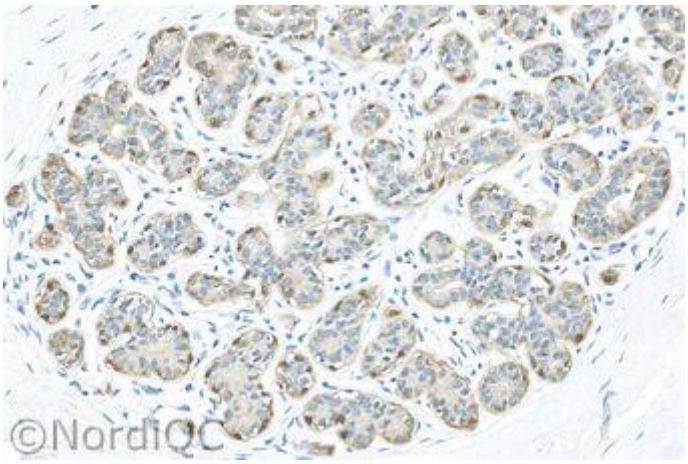


Fig 3b
Insufficient staining for S100 of the breast hyperplasia using same protocol as in Figs. 1b & 2b., same field as in Fig. 3a. The myoepithelial cells show a weak and equivocal staining reaction also compare with Fig. 4b, same protocol.

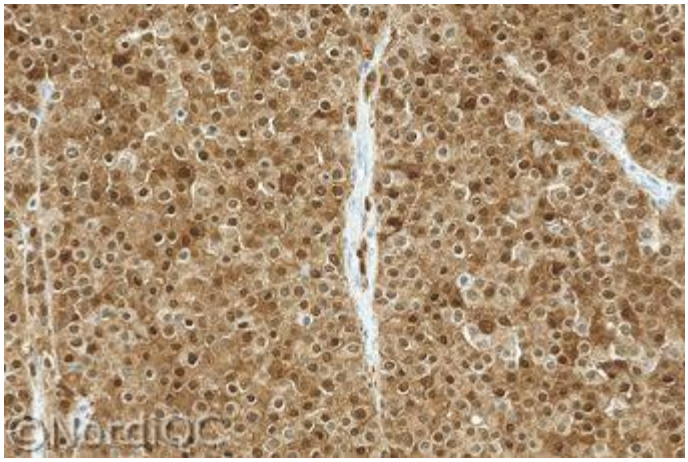


Fig 4a
Optimal staining for S100 of the malignant melanoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic and nuclear staining reaction.
No background reaction is seen.

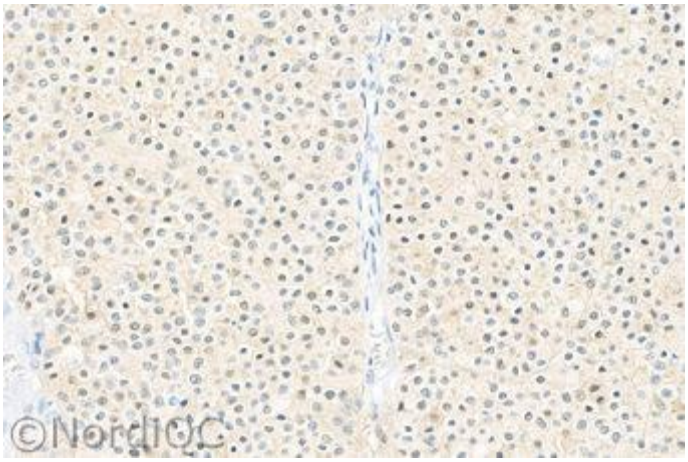


Fig 4b
Insufficient staining for S100 of the malignant melanoma using same protocol as in Figs. 1b – 3b., same field as in Fig. 4a.
The neoplastic cells show a weak and equivocal staining reaction.

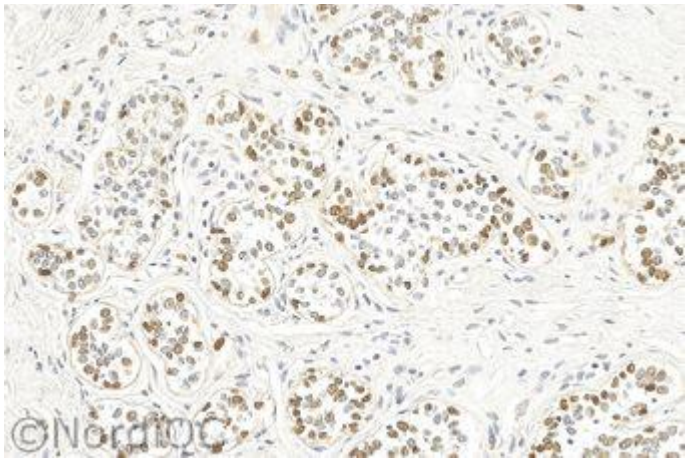


Fig 5a
Insufficient staining for S100 of the breast hyperplasia using excessive proteolytic pre-treatment. The cytoplasmic compartment of both the myoepithelial cells and the glandular epithelial cells is digested and only the nuclei are left and demonstrated. Also compare with Fig. 5b, same protocol.

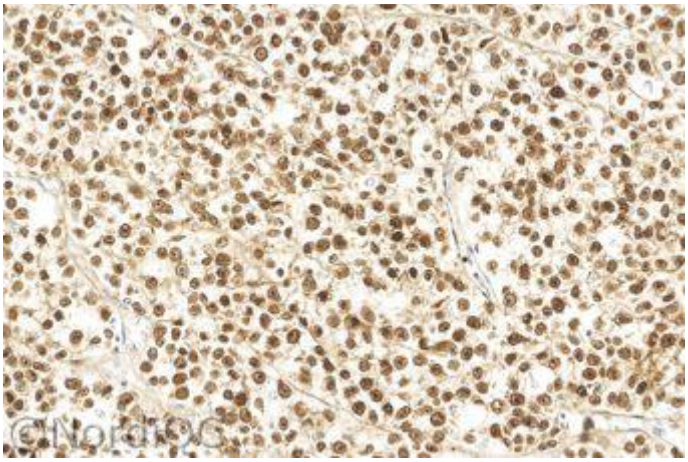


Fig 5b
Insufficient staining for S100 of the malignant melanoma using excessive proteolytic pre-treatment, same protocol as in Fig. 5a.
The cytoplasmic compartment is digested and only the nuclei are left in the neoplastic cells.

SN/MV/LE 29-03-2012