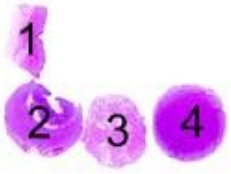
Assessment Run 20 2007

S-100 protein (S100)

The slide to be stained for S100 comprised:
1. Skin, 2. Appendix, 3. Breast, 4. Malignant melanoma.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a 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 and satellite cells in the muscularis propria and submucosa in the appendix. The epithelial cells and muscle cells should be negative.
- A moderate to strong, distinct nuclear and cytoplasmic staining 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 of the majority of the neoplastic cells of the metastatic malignant melanoma.
- A strong, distinct nuclear and cytoplasmic reaction of fat cells and macrophages in all specimens.

106 laboratories submitted stains. At the assessment, 35 obtained an optimal result (33%), 44 good (42%), 25 borderline (24%) and 2 poor (2%).

The following Abs were used:
mAb clone **4C4.9** (Ventana, n=2; NeoMarkers, n=1)
mAb clones **15E2E2** (BioGenex, n=1; DCS, n=1)
pAb **18-0046** (Zymed, n=1)
pAb **760-2523** (Ventana, n=3)
pAb **MS-296-P** (NeoMarkers, n=1)
pAb **RB-9081-P** (NeoMarkers, n=1)
pAb **Z0311** (Dako, n=95)

Mandatory for an optimal result in this assessment was the use of pAb Z0311 (35 out of 96 laboratories; 37%) using following protocol settings:
Both Heat Induced Epitope Retrieval (HIER) and enzymatic pre-treatment. Using HIER 24 out of 57 laboratories obtained an optimal result (42%), using enzymatic pre-treatment 10 out of 28 laboratories obtained an optimal result (36 %). Among 11 laboratories omitting pre-treatment, one obtained an optimal result (9%).
With HIER 17 laboratories used Tris-EDTA/EGTA pH 9, 3 Ventana Cell Conditioning 1, 2 citrate buffer pH 6.0 and one Dako TRS-buffer pH 6.1.
With enzymatic pre-treatment 4 laboratories used Ventana protease 1, 4 used Dako proteinase K and 2 used protease type XXIV.
The pAb Z0311 was typically diluted in the range of 1:800 – 1:8,000 depending on the total sensitivity of the protocol employed.
Using these protocol settings 75 out of 90 laboratories (83%) produced a sufficient staining (optimal or good).

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

Virtually all laboratories were able to demonstrate S-100 in the malignant melanoma, whereas the prevalent feature of an insufficient staining was a general too weak or false negative staining of the normal melanocytes, the Langerhans cells and the myoepithelial cells. HIER and enzymatic pre-treatment seemed nearly equally useful to obtain an optimal result. However, differences in the staining patterns were seen with these two pre-treatment methods: Using HIER, the melanoma cells were uniformly stained, while using enzymatic pre-treatment the staining showed a more heterogeneous pattern and in several cases the cytoplasmic compartment was totally

extracted due to an excessive digestion.

With HIER and pAb Z0311 50 out of 57 (88%) laboratories obtained a sufficient result, while 19 out of 28 laboratories (68%) using enzymatic pre-treatment and pAb Z0311 obtained a sufficient result.
Omission of epitope retrieval typically resulted in a general too weak reaction and 6 of 11 laboratories using this procedure received an insufficient mark.

S-100 was also assessed in Run 7 2003, in which 63 laboratories participated. The overall proportion of sufficient staining increased from 71 % in run 7 to 75% in the present run (however, the number of participants doubled from run 7 to run 20, so for many of the laboratories, this was the first time to stain for S-100). The proportion of poor results decreased from 13% in run 7 to 2% in run 20 while borderline staining increased from 16% to 24%.

Skin seemed to be a good and useful control for S-100, as all laboratories with optimal staining of the other tissues could demonstrate melanocytes and Langerhans cells.

Conclusion
The pAb **Z0311** appears to be a robust and good marker for S-100. HIER should be the preferred pre-treatment. Skin is an appropriate control: the melanocytes and the Langerhans cells must show a strong and distinct reaction, while the squamous epithelial cells shall remain negative.
Insufficient results seem in many laboratories to be due to lack of awareness of optimal positive and negative control material giving difficulties in finding the right pre-treatment and dilution of the primary Ab.

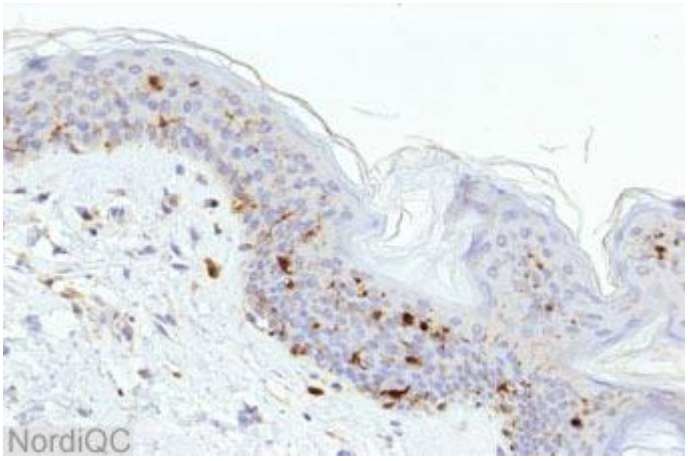


Fig. 1a
Optimal staining of S-100 in the skin specimen using HIER pretreatment. The melanocytes and the Langerhans cells are clearly demonstrated in the epidermis, while the epithelial cells are negative.

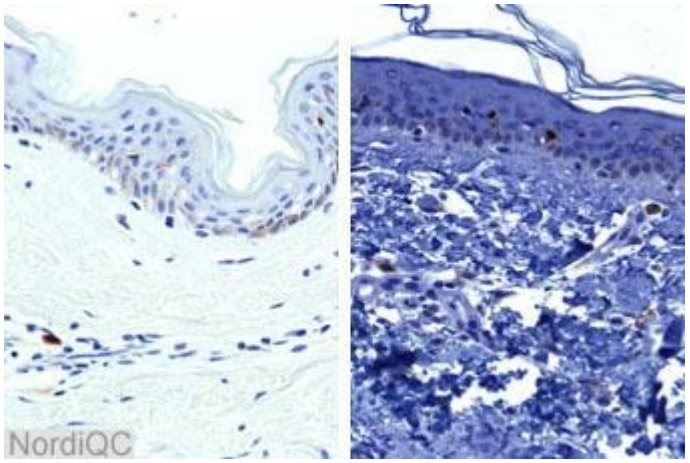


Fig. 1b
Left: Insufficient staining of S-100 in the skin specimen. The Langerhans cells and melanocytes are only faintly stained.
Right: Insufficient staining of S-100 in the skin specimen – the combination of a too strong haematoxylin staining and a weak staining for S100 obscures the interpretation.

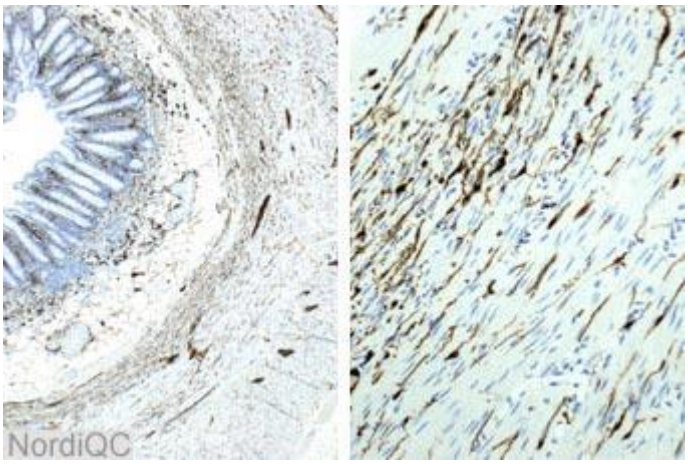


Fig. 2a
Left: Optimal staining of S-100 protein in a normal appendix using enzyme pretreatment. An intense reaction of macrophages, nerve fibers and fat cells are seen in the lamina propria and ganglion cells and nerve fibers (Schwann cells) are clearly demonstrated in the muscularis propria.
Right: High magnification of the strong reaction of ganglion cells and nerve fibers (Schwann cells) in the muscularis propria, while the muscle cells are negative.

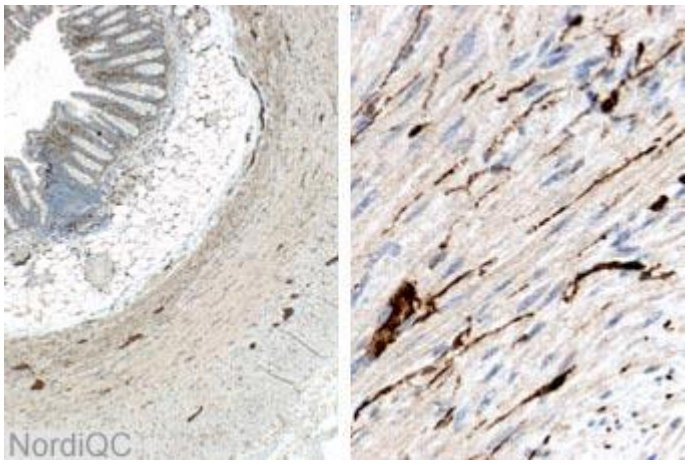


Fig. 2b
Left: Staining of S-100 protein in a normal appendix using an insufficient protocol. The cells expected to stain are demonstrated, however also a high unspecific background reaction is seen, due to a too high concentration of the primary Ab.
Right: High magnification of the reaction in the muscularis propria, where the muscle cells show a false positive reaction.

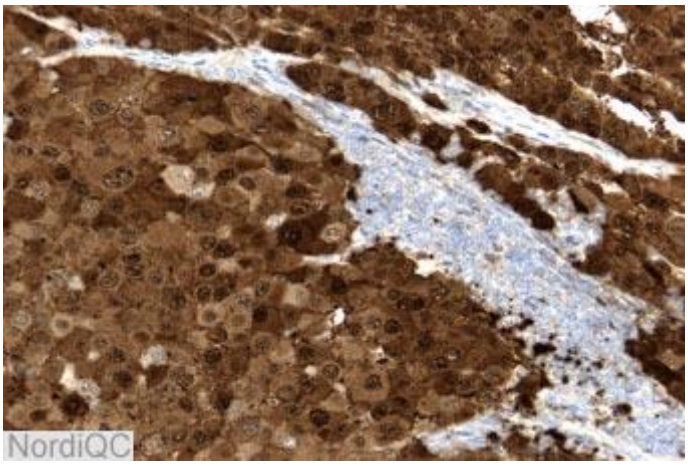


Fig. 3a
Optimal staining for S100 of the melanoma. Virtually all the neoplastic cells show a strong nuclear and cytoplasmic staining using HIER as pre-treatment.

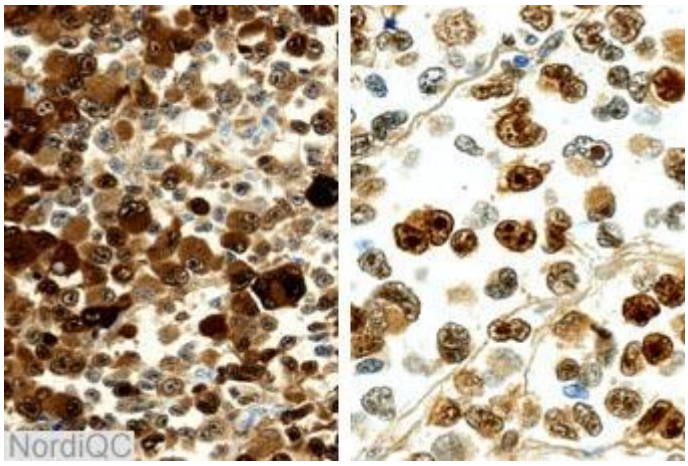


Fig. 3b
Left: Optimal staining for S100 of the melanoma. Virtually all the neoplastic cells show a strong nuclear and cytoplasmic staining using proteolytic pre-treatment. However the reaction pattern is more heterogeneous compared to Fig. 3a.
Right: Insufficient staining for S100 using excessive proteolytic pre-treatment. The cytoplasmic compartment is digested and only the nuclei are left in the neoplastic cells.

AL/SN/MV/LE 2-7-2007