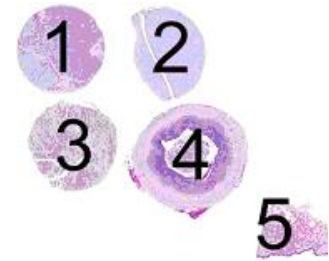


The slides to be stained for S100 comprised:

1: malignant melanoma (small intestine), 2: brain, 3: malignant melanoma (testis), 4: appendix, 5: blue nevus.



Criteria for assessing a staining as optimal included: strong and distinct nuclear and cytoplasmic staining reaction in the tumor cells of the malignant melanomas and the blue naevus. Glial cells, Schwann cells, fat cells, and reticulum cells including epidermal Langerhans cells should also be demonstrated.

63 laboratories submitted stainings. At the assessment, 19 obtained an optimal result (30%), 26 good (41%), 10 borderline (16%) and 8 poor (13%)

55 used pAb Z0311 (DakoCytomation), 3 pAb 760-2523 (Ventana), 1 pAb NCL-S100p (Novocastra), 1 pAb (BioMeda), 1 mAb DAK-S100B/2 (DakoCytomation), 1 mAb 15E2E2 (BioGenex), and 1 mAb S1/61/69 (Novocastra).

Mandatory for an optimal result in this assessment was the use of pAb Z0311. Pre-treatment was used for all but one of the optimal stainings. The protocol without pretreatment used prolonged incubation time and a high Ab concentration. HIER and proteolytic pre-treatment seemed equally useful. However, slight differences in the staining patterns were seen with these two pre-treatment methods: Using HIER, the dendritic cell in the germinal centres were stained, while they were unstained after proteolytic pretreatment.

Most protocols gave a good staining of the malignant melanomas, while only the optimal protocols gave a proper staining of the blue naevus.

The most frequent causes of insufficient stainings (often in combination) were:

- Inappropriate Ab
- A too low primary Ab concentration
- A too high primary Ab concentration after epitope retrieval
- Lack of pre-treatment
- Excessive proteolytic pre-treatment.

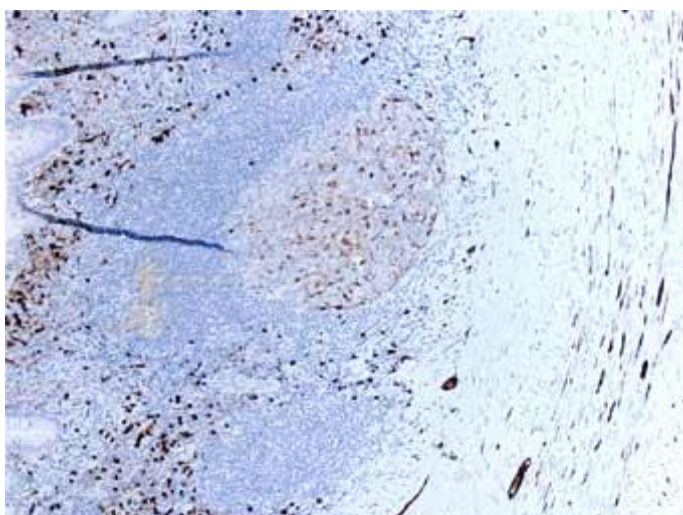


Fig. 1a
Optimal staining of S-100 protein in a normal appendix using HIER. An intense staining is seen of macrophages in the lamina propria, dendritic reticulum cells in the germinal center, and ganglion cells in the muscularis propria.

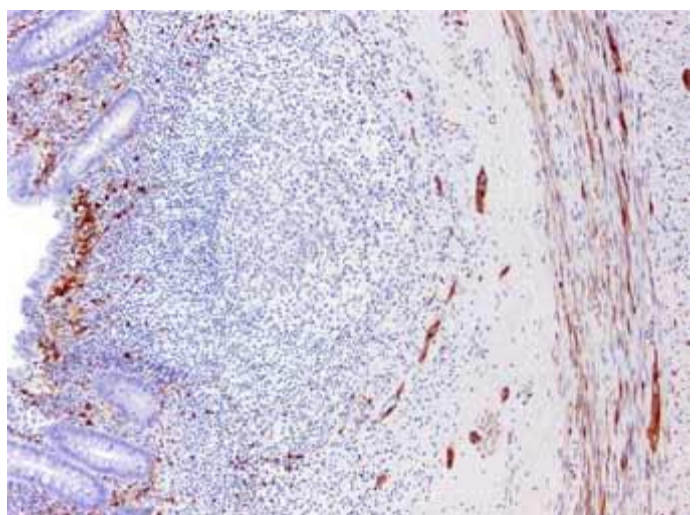


Fig. 2a
Optimal staining of S-100 protein in a normal appendix using proteolytic pretreatment (same field as in Fig. 1a). The same cells are stained as after HIER with the exception of the dendritic reticulum cells.

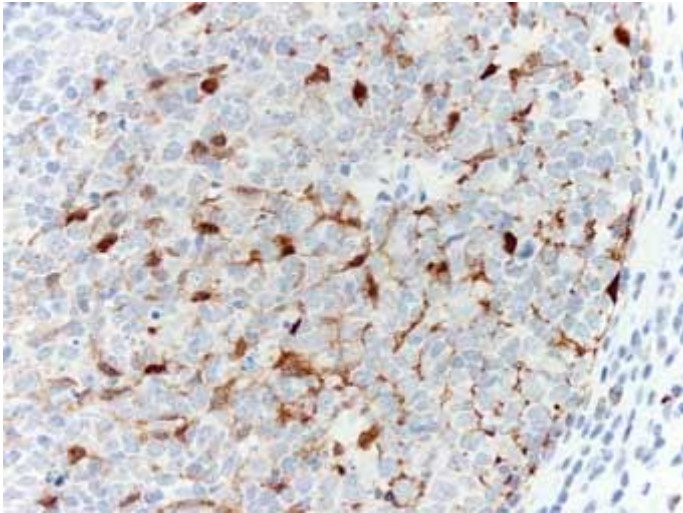


Fig. 1b
Optimal staining of S-100 protein in a normal appendix using HIER. Higher magnification of Fig. 1a to show the dendritic reticulum cells.

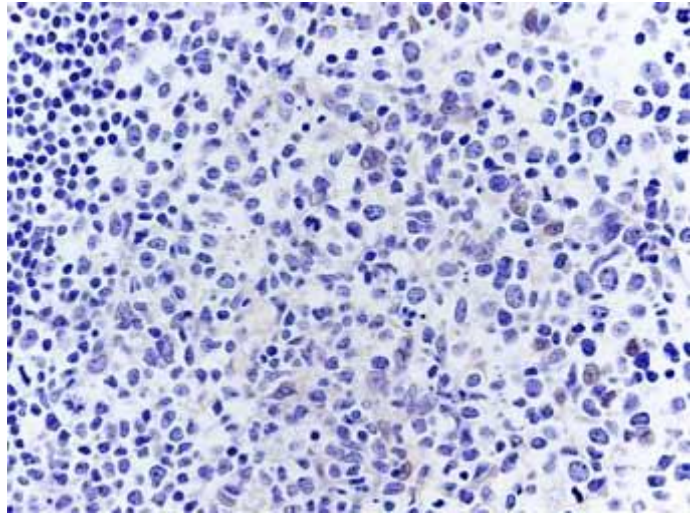


Fig. 2b
Optimal staining of S-100 protein in a normal appendix using proteolytic pre-treatment. Higher magnification of Fig. 2a. The dendritic reticulum cells are unstained. (same field as in Fig. 1b)

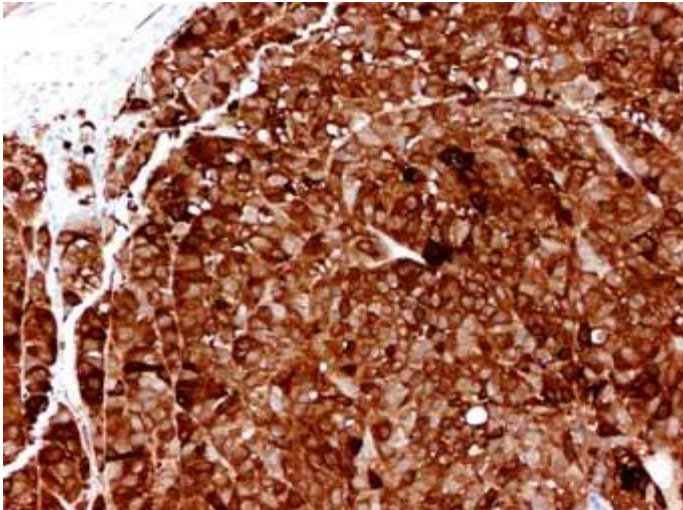


Fig. 1c
Optimal staining of S-100 in a malignant melanoma using HIER.

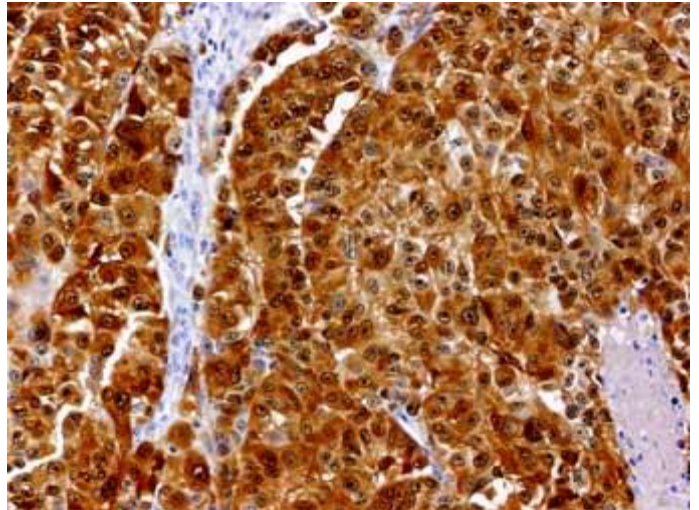


Fig. 2c
Optimal staining of S-100 in a malignant melanoma using proteolytic pre-treatment (same field as in Fig. 1c).

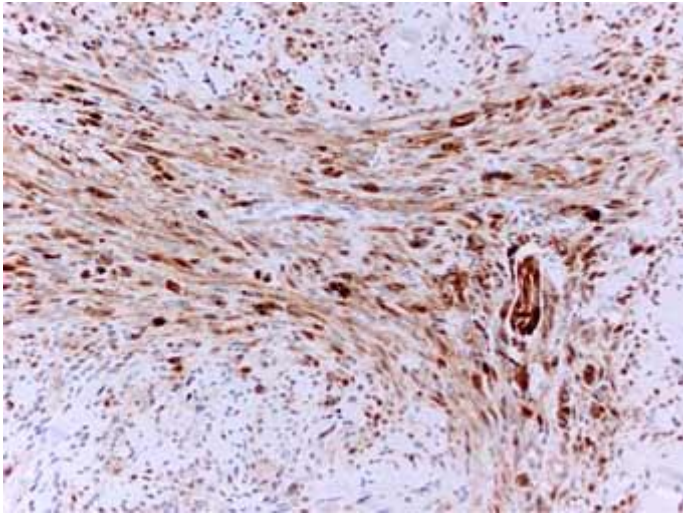


Fig. 1d
Optimal staining of S-100 in a blue naevus using HIER.

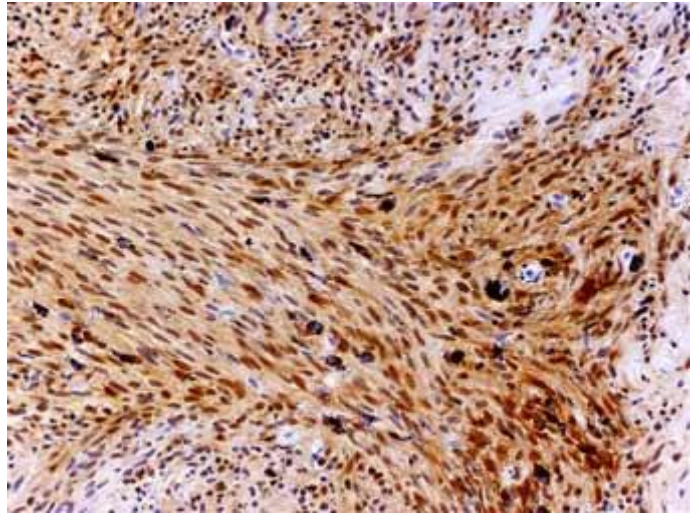


Fig. 2d
Optimal staining of S-100 in a blue naevus using proteolytic pre-treatment (same field as in Fig. 1d).

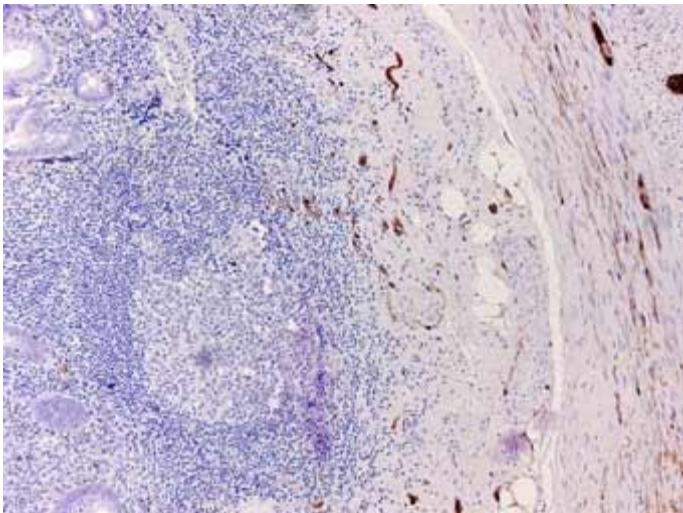


Fig. 3a
Staining of S-100 in an appendix using an insufficient protocol (no pre-treatment). Compare with Fig. 3b. ##

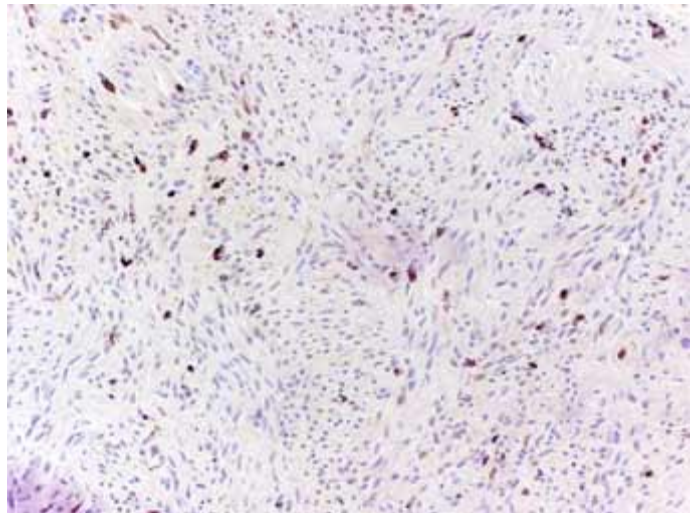


Fig. 3b
Staining of S-100 in a blue naevus using an insufficient protocol (no pre-treatment). Compare with Fig 1d and 2d (same field).

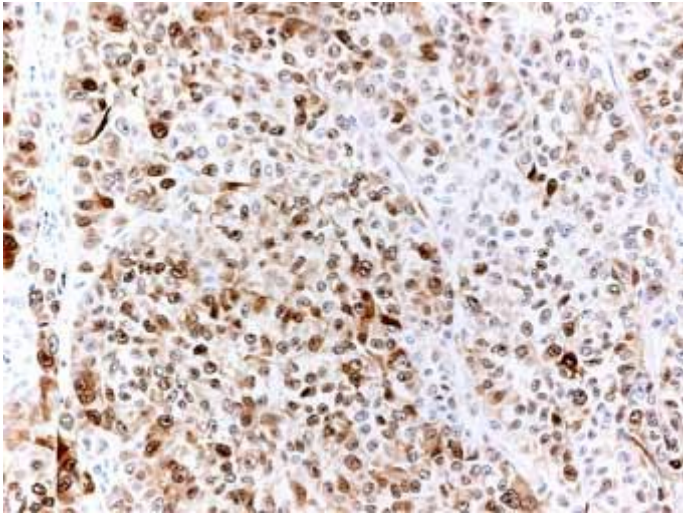


Fig. 4a
Staining of S-100 in a malignant melanoma using an insufficient protocol (too much proteolytic digestion). The cytoplasm of the tumour cells appear empty (same field as in Fig. 1c and 2c).

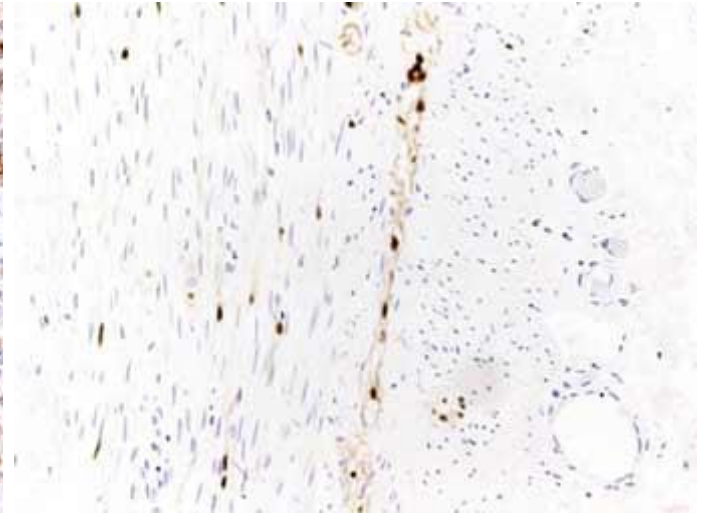


Fig. 4b
Staining of S-100 in an appendix using an insufficient protocol (too much proteolytic digestion). The ganglion cells appear empty.

SN/MV/LE 20-8-2003