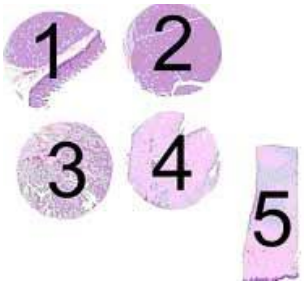
Assessment Run 7 2003

Melan A (MLA)



The slides to be stained for Melan-A comprised:
1: malignant melanoma (small intestine), 2: granulosa cell tumour (ovary),
3: malignant melanoma (testis), 4: adrenal gland, 5: blue nevus.

Criteria for assessing a Melan-A staining as optimal included: a strong and distinct intracytoplasmic reaction in the normal melanocytes and the tumour cells of the malignant melanoma and the blue naevus. If mAb A103 was used, also a distinct staining of the granulosa cell tumour and adrenal cortical cells should be seen. A weak non-specific staining of enterocytes is accepted. All other cells should remain unstained.

35 laboratories submitted stainings. At the assessment, 14 laboratories achieved optimal staining (40%), 10 good (29%), 8 borderline (23%), and 3 poor staining (8%).

33 used mAb A103 (DakoCytomation (24), Novocastra (7), Ventana (2)). 2 laboratories used mAb Melan A Ab-3 (Neomarkers), a cocktail of the two clones M2-7C10 and M2-9E3. Optimal results could be obtained with both mAb A103 and the melan-A Ab3 cocktail.

Using mAb A103, it was mandatory to use HIER in Tris-EDTA/EGTA pH 9 to obtain an optimal staining. While melanocytes and the malignant melanoma could in most cases be stained with less sensitive protocols, the granulosa cell tumour and the adrenal cortex could not. The optimal dilution for A103 ranged from 1:25 to 1:200.

Using the mAb cocktail M2-7C10 and M2-9E3 an optimal result was obtained with HIER in Citrate pH 6 (one laboratory).

The most frequent causes of insufficient stainings (often in combination) were:
- A too low concentration of the primary Ab
- No or insufficient HIER (citrate pH 6 or too short heating time with Tris-EDTA/EGTA pH 9)

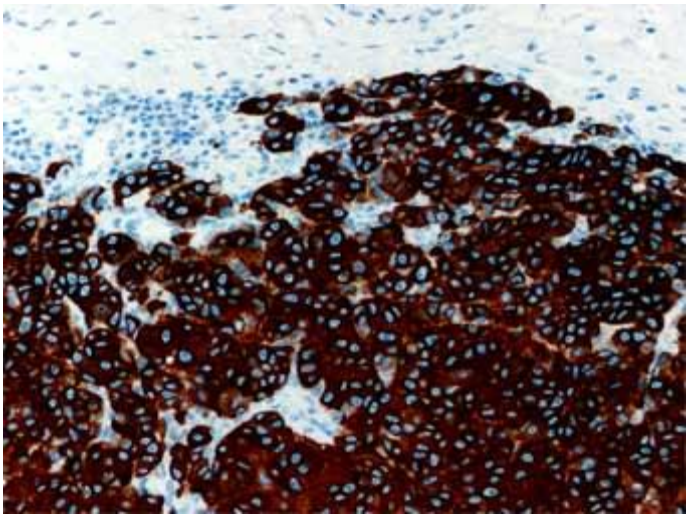


Fig. 1a
Optimal staining of melan-A (mAb A103) in a malignant melanoma infiltrating the small intestine.

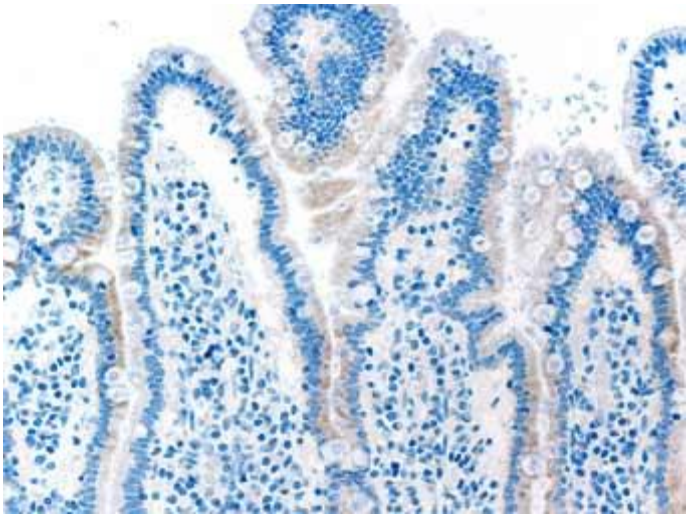


Fig. 1b
Staining for melan-A (mAb A103). A faint, probably non-specific staining of enterocytes could not be avoided in protocols otherwise giving optimal results.

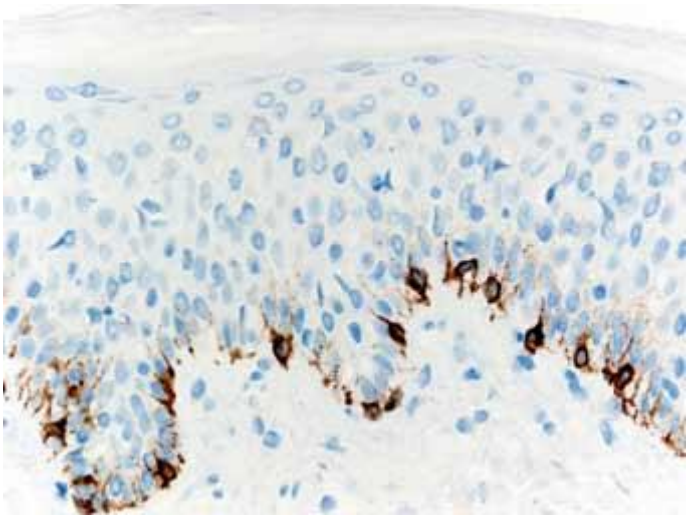


Fig. 1c
Optimal staining of melan-A (mAb A103) in normal melanocytes in the epidermis.

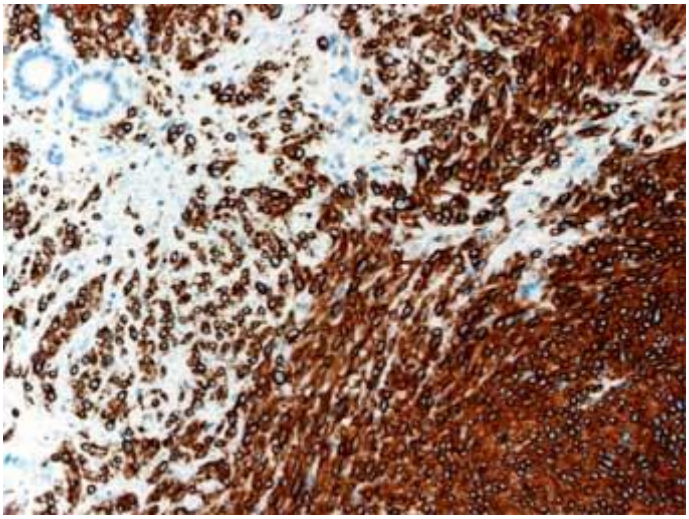


Fig. 1d
Optimal staining of melan-A (mAb A103) in a blue naevus.

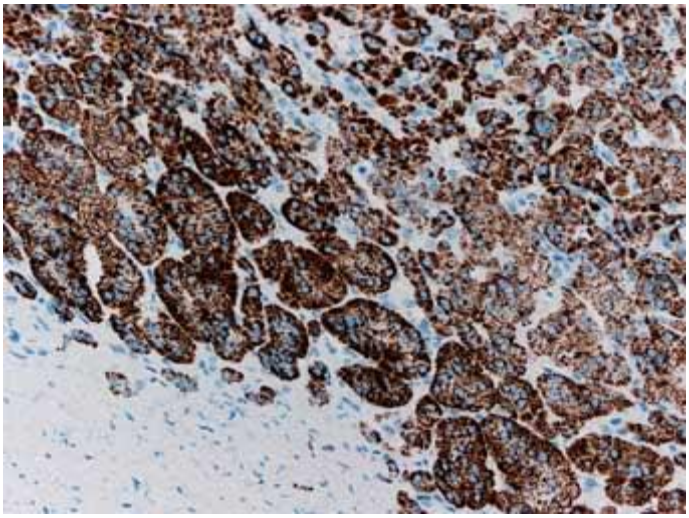


Fig. 1e
Optimal staining of a normal adrenal cortex using of mAb A103. A strong positivity of all epithelial cells is seen.

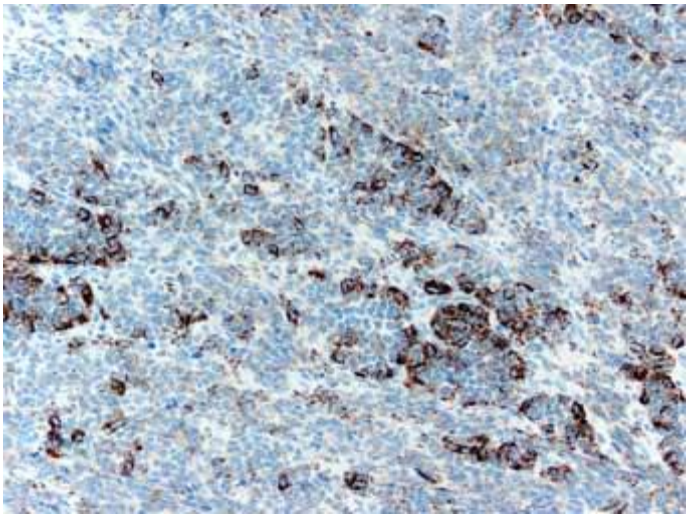


Fig. 1f
Optimal staining of a granulosa cell tumour using mAb A103. A heterogenous positivity is seen.

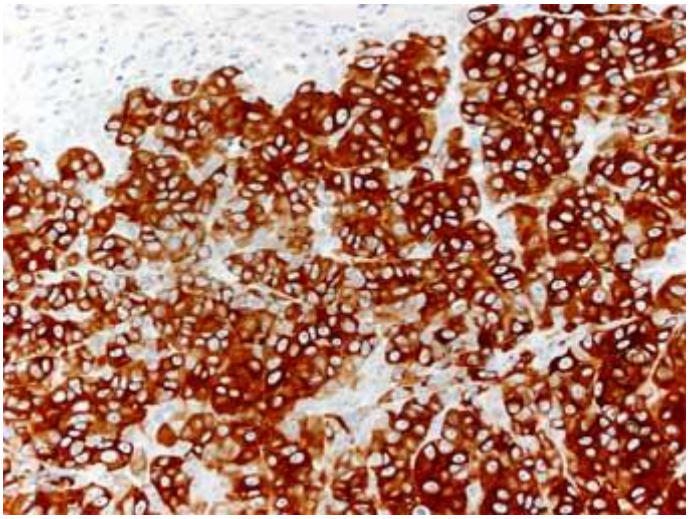


Fig. 2a
Staining of melan-A (mAb A103) in a malignant melanoma infiltrating the small intestine, using an insufficient protocol. Same field as in Fig. 1a. The tumour cells are strongly stained. However, compare Figs. 2b and 2c.

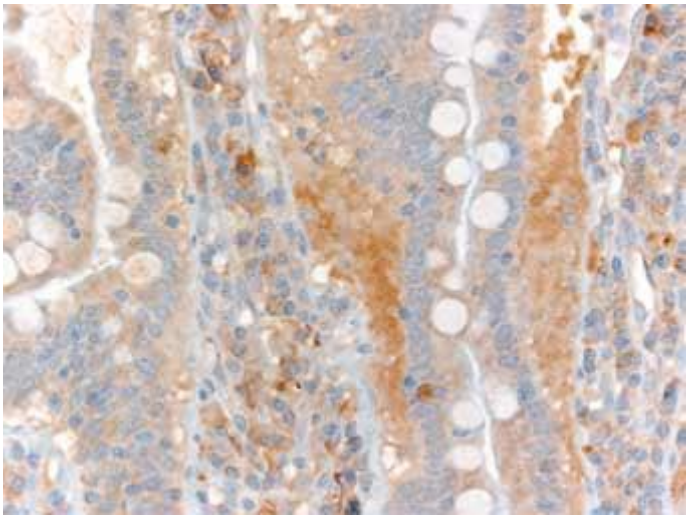


Fig. 2b
Staining for melan-A (mAb A103) using an insufficient protocol. A moderate non-specific staining of enterocytes and plasma cells is seen.

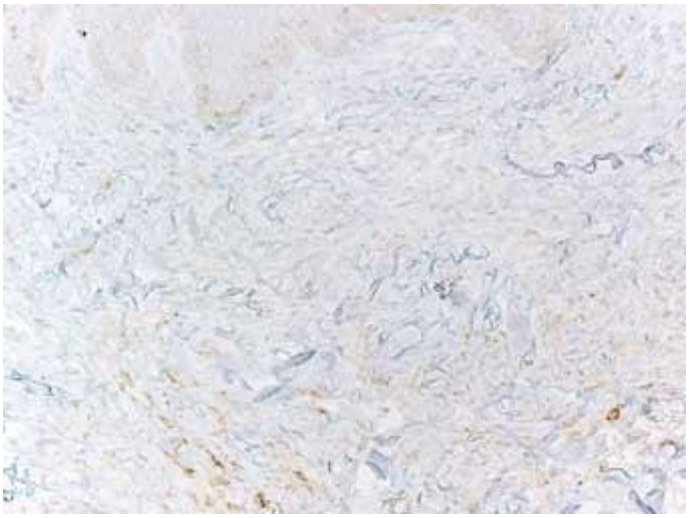


Fig. 2c
Staining of melan-A (mAb A103) in a blue naevus, using an insufficient protocol. The melanocytes and tumour cells are weakly stained. The field corresponds that of Figs. 1c-d.

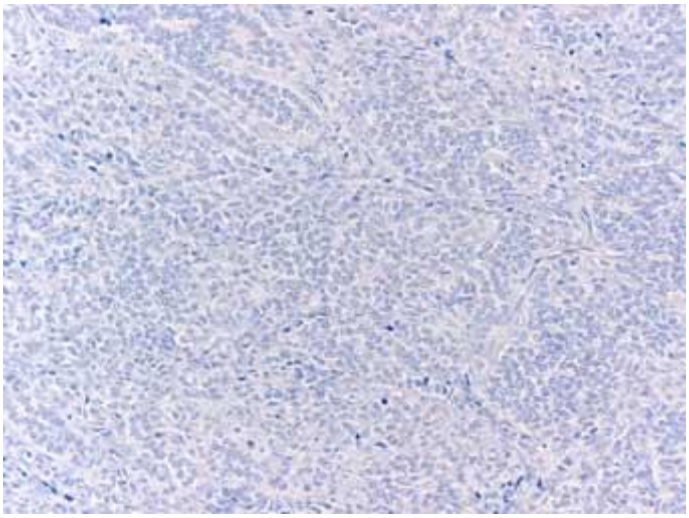


Fig. 2d
Staining of a granulosa cell tumour using mAb A103 using an insufficient protocol. The tumour cells are unstained. Same field as in Fig. 1f.

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