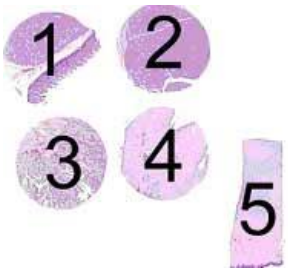
Assessment Run 7 2003

Melanosoma specifik antigen (MSA)

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



Criteria for assessing an MSA staining as optimal included: a strong and distinct intracytoplasmic reaction in the tumour cells of the malignant melanomas and the blue naevus (which expressed scarce amounts of MSA compared to the melanomas), while no staining reaction should be seen in other cells.

66 laboratories submitted stainings. At the assessment, 30 laboratories achieved optimal staining (45%), 19 good (29%), 13 borderline (20%), and 4 poor staining (6%).

All used mAb HMB45, which was obtained from DakoCytomation (53), Enzo (6), Ventana (5), and Novocastra (2).

Mandatory for an optimal staining was a protocol based on a proper dilution of the primary Ab in combination with an efficient HIER and a non-biotin based detection system or an efficient biotin blocking (if using a biotin based detection system).

Among 9 using a ready-to-use (RTU) primary antibody, only one got an optimal result. Laboratories using Tris-EDTA/EGTA and Citrate heating buffers got almost equal results. However, among protocols without HIER only one of 8 gave an optimal result.

The most frequent causes of insufficient stainings (often in combination) were:
- A too low concentration of the primary Ab.
- An RTU Ab.
- No or insufficient HIER.
- Proteolytic pre-treatment (often resulting in a weak demonstration of MSA as well as a false positive staining of plasma cells).
- Use of a biotin based detection system without suppression of endogenous biotin.

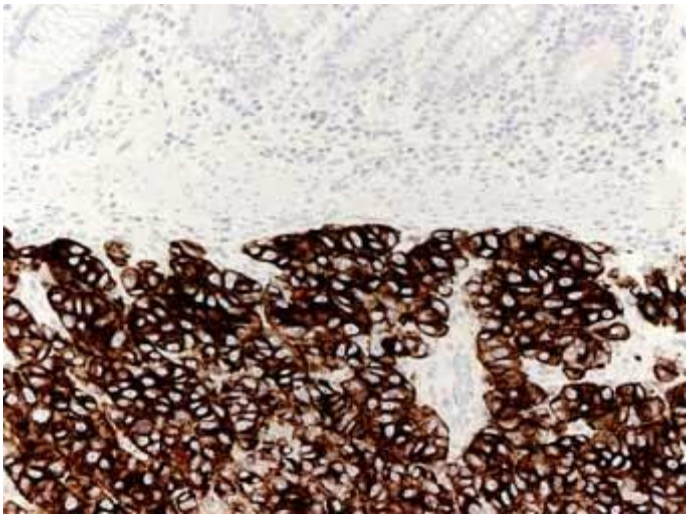


Fig. 1a
Optimal staining of MSA in a malignant melanoma infiltrating the small intestine. All tumour cells are strongly stained. No background staining.

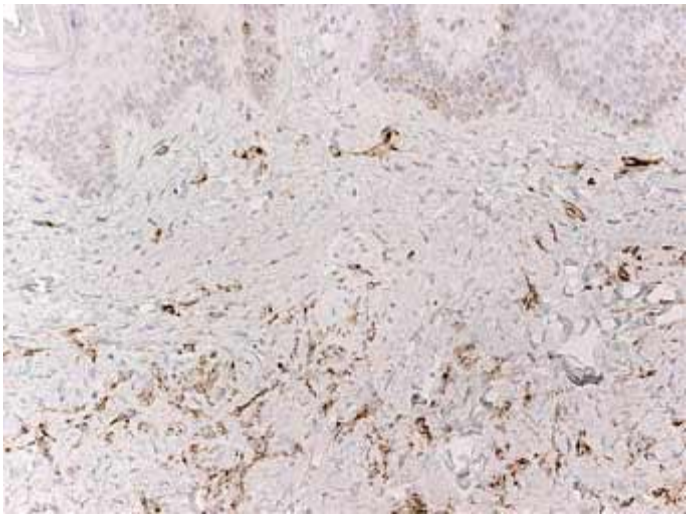


Fig. 1b
Optimal staining of MSA in a blue naevus. Most tumour cells are moderately stained. In the basal epidermis, some melanin is seen.

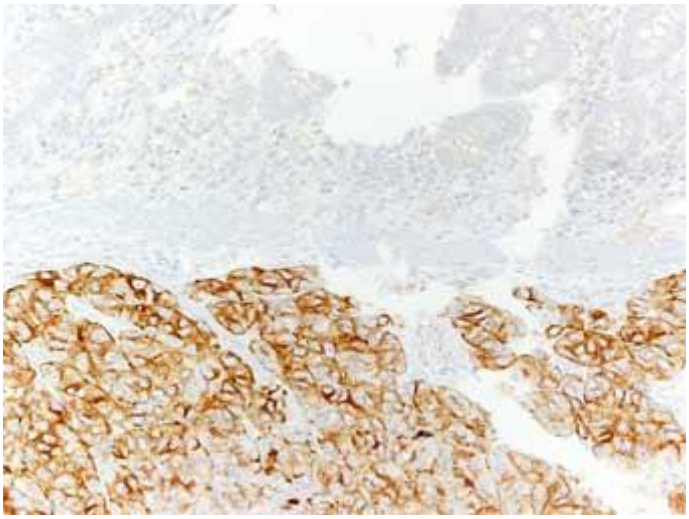


Fig. 2a
Staining of MSA in a malignant melanoma infiltrating the small intestine (same field as in Fig. 1a), using an insufficient protocol. The tumour cells are moderately stained. There is a slight staining of enterocytes. Also compare with Fig. 2b and 2c.

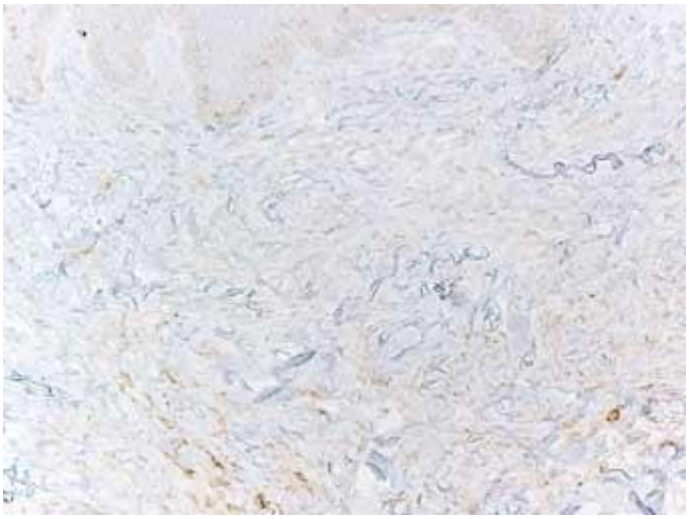


Fig. 2b
Staining of MSA in a blue naevus, using an insufficient protocol. The tumour cells are weakly stained (same field as in Fig 1b).

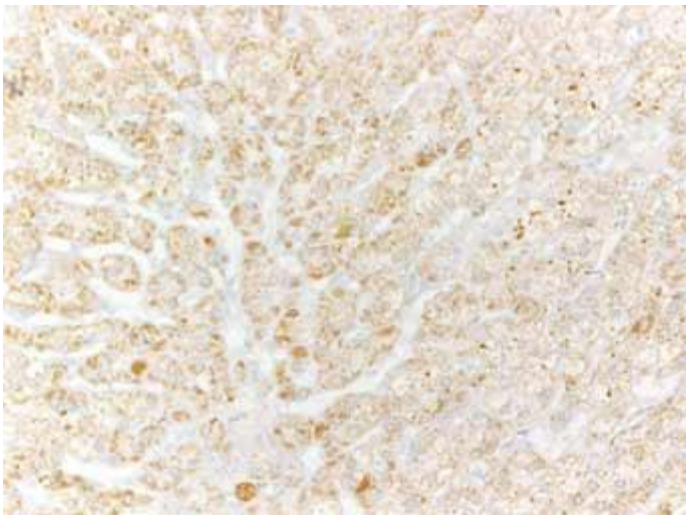


Fig. 2c
Staining for MSA in a normal adrenal gland using an insufficient protocol. A false positive staining of the epithelial cells are due to endogenous biotin (which was not suppressed in this biotin based protocol). Compare with Fig. 3.

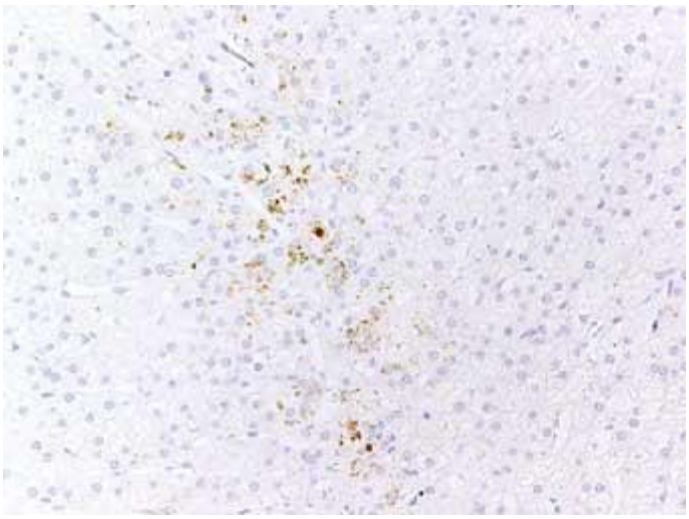


Fig. 3
Appropriate staining for MSA in a normal adrenal gland. Some lipofuscin is seen, otherwise the cells are unstained.

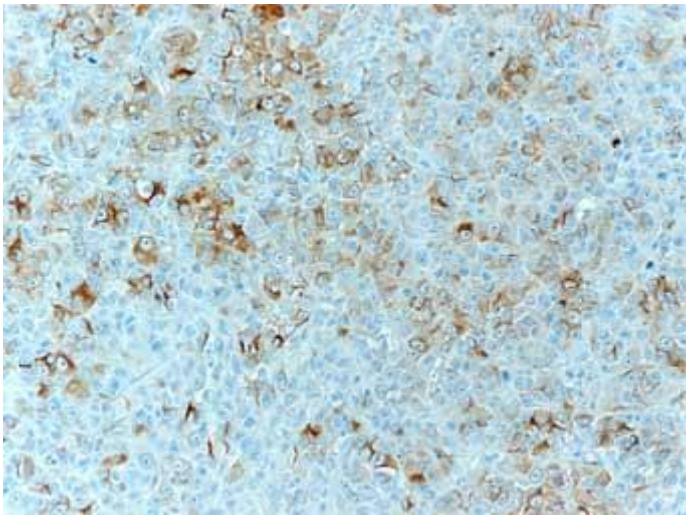


Fig. 4a
Staining for MSA in a malignant melanoma infiltrating the small intestine (same field as in Fig. 1a), using an insufficient protocol (demasking was omitted). Many tumour cells are unstained.

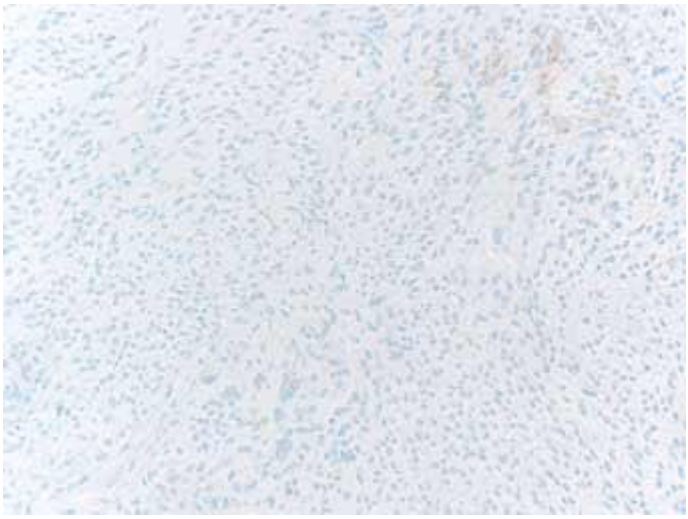


Fig. 4b
Staining for MSA in a blue naevus. The tumor cells are almost unstained (same field as in Fig 1b), using an insuffcient protocol (same as Fig. 4a).

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