The slide to be stained for Melan A comprised:
1. Desmoplastic malignant melanoma, 2. Blue naevus, 3. Adrenal gland,
All specimens were fixed in 10 % NBF.

Criteria for assessing a Melan A staining as optimal included:

- A strong, distinct cytoplasmic staining of the normal melanocytes, the blue naevus and the malignant melanoma.
- A distinct cytoplasmic staining of scattered cells in the desmoplastic malignant melanoma.
- A strong, distinct granular cytoplasmic staining in virtually all adrenal cortical cells (clone A103 only).
- A distinct cytoplasmic staining of the majority of cells in the granulosa cell tumour (clone A103 only).

79 laboratories submitted stains. At the assessment 10 achieved optimal marks (13 %), 15 good (19 %), 24 borderline (30 %) and 30 (38 %) poor marks.

The following Abs were used:
- mAb clone A103 (Dako, n=58; Novocastra, n=8; NeoMarkers, n=3; Ventana, n=3; Monosan, n=2; BioGenex, n=1)
- mAb clones M2-7C10 + M2-9E3 (NeoMarkers, n=3)
- mAb clone M2-7C10 (Cell Marque, n=1)

In this assessment an optimal stain could only be obtained using the mAb clone A103. All protocols giving optimal results were based on HIER using a Tris-EDTA/EGTA buffer pH 9. 10 out of 49 (20 %) laboratories using this obtained optimal marks. In these protocols clone A103 typically was diluted in the range of 1:20 - 1:50 depending on the total sensitivity of the protocol employed. Among 33 stains using Tris-EDTA/EGTA and A103 in a dilution of 1:50 or stronger, 21 (64%) obtained a sufficient result (optimal and good).

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Less successful primary Ab
- Less successful ready-to-use (RTU) mAb clone A103 (all of four stains submitted were insufficient)
- HIER with other buffers than Tris-EDTA/EGTA pH 9

In the assessment the prevalent feature of an insufficient staining was a false negative reaction of the desmoplastic melanoma. In general, almost all laboratories (90 %) were able to detect Melan A in the blue naevus and the metastatic malignant melanoma. However, in a diagnostic setting the capability to detect Melan-A can be crucial for the identification of the melanocytic lineage of a spindle cell tumour. This assessment stresses that a highly sensitive IHC system is mandatory in such cases. Using clone A103 the insufficient staining of the desmoplastic melanoma typically was accompanied with a too weak or even false negative reaction in the granulosa cell tumour and weak staining of the adrenal zona reticularis.

An applicable quality indicator using the clone A103 was the ability to demonstrate a strong granular cytoplasmic reaction in virtually all the epithelial cells throughout the adrenal cortex. Using another clone such as M2-7C10 (which does not react with steroid producing cells) the reaction should be as strong as possible in melanocytes and melanocytic tumour cells without background reaction.

Melan A was also assessed in Run 7, in which 35 laboratories participated. Out of these 31 % (11 laboratories) had an insufficient staining. Each laboratory was given specific recommendations to improve their protocol. 9 laboratories, which obtained an insufficient result in run 7 submitted a new Melan A stain in run 16. 4 out of these followed the recommendation and 1 of them (25 %) improved the mark from insufficient to good. 5 laboratories did not follow the recommendations and none of these improved their marks.

The overall proportion of insufficient staining was in this run increased from 31 % in run 7 to 68 % in run 16. This marked increase is probably due to the tissue material circulated and is not an indicator of a declining performance in the laboratories. Almost 90 % were capable to demonstrate Melan A in the blue naevus and
metastatic malignant melanoma, but in the Run 16 assessment a desmoplastic melanoma was included and this tumour appeared to be the discriminator between a sufficient and an insufficient protocol.

**Conclusion**
- The mAb clone **A103** seems to be the most sensitive marker for MLA.
- HIER in an alkaline buffer such as Tris-EDTA/EGTA pH 9 is highly recommended for optimal performance.
- The Ab concentration has to be calibrated to give the strongest possible reaction in cells with a low expression of the epitope in order to be able to detect Melan A in e.g., desmoplastic malignant melanoma.

![Fig. 1a](image1) Optimal staining for Melan A of the blue nevus. All the neoplastic cells and the normal melanocytes show a strong cytoplasmic staining with no background reaction.

![Fig. 1b](image2) Staining for Melan A in the blue nevus using an insufficient protocol (same field as in Fig. 1a.). The cells expected to cells are demonstrated. However, compare with Fig. 2b – same protocol.

![Fig. 2a](image3) Optimal staining for Melan A of the desmoplastic melanoma. The majority of the neoplastic cells show a distinct, dot-like reaction (same protocol used in Fig. 1a).

![Fig. 2b](image4) Insufficient staining Melan A of the desmoplastic melanoma (same field as in Fig 2a). All the neoplastic cells are virtually negative (same protocol used in Fig. 1b).
Fig. 3a
Optimal staining for Melan A (clone A103) of the adrenal gland. All the epithelial cells, including zona reticularis demonstrated (same protocol used in Fig. 1a and 2a).

Fig. 3b
Staining for Melan A (clone A103) of the adrenal gland using an insufficient protocol (same protocol used in Fig. 1b and 2b). Only the epithelial cells in zona glomerulosa show a moderate positive staining, while zona reticularis is negative (same field as in Fig. 3a).

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