Assessment Run 16 2006

Melan A (MLA)

The slide to be stained for Melan A comprised:
1. Desmoplastic malignant melanoma, 2. Blue naevus, 3. Adrenal gland,
4. Granulosa cell tumour, 5. Metastatic malignant melanoma.
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 nevus 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 nevus 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 be to 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.

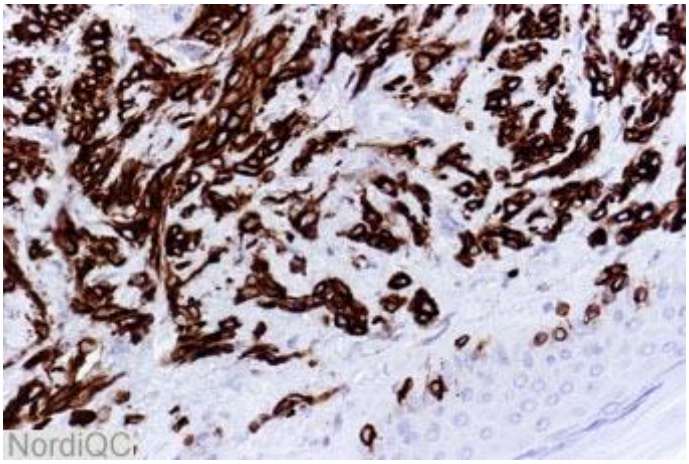

Fig. 1a
Optimal staining for Melan A of the blue nevus. All the neoplastic cells and the normal melanocytes show a strong cytoplasmic staining with no background reaction.

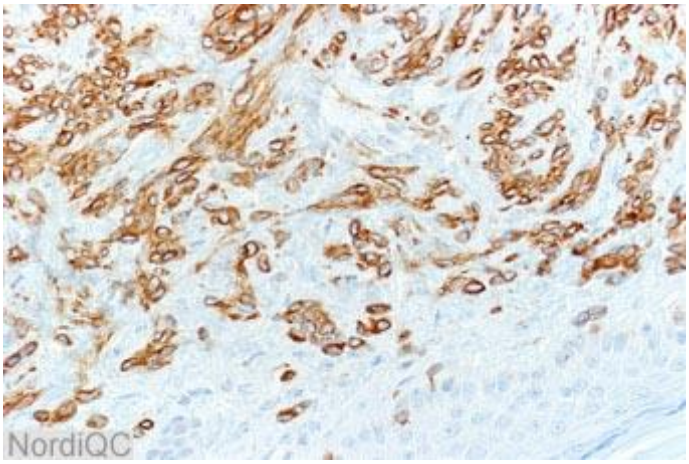

Fig. 1b
Staining for Melan A in the blue nevus using an insufficient protocol (same field as in Fig. 1a.). The cells expected to cells are demonstrated. However, compare with Fig. 2b – same protocol.

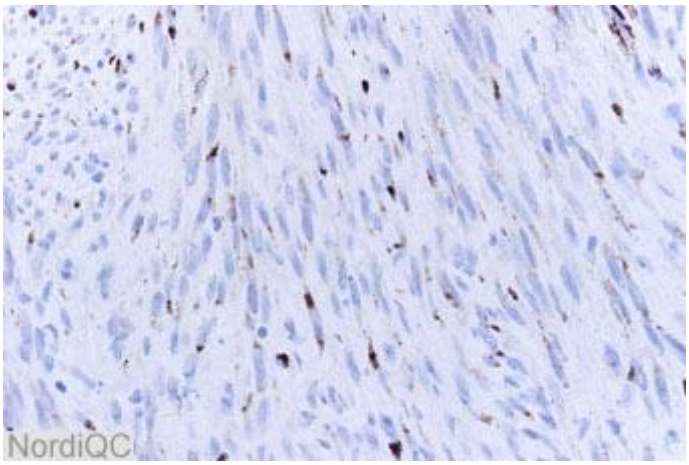

Fig. 2a
Optimal staining for Melan A of the desmoplastic melanoma. The majority of the neoplastic cells show a distinct, dot-like reaction (same protocol used in Fig. 1a).

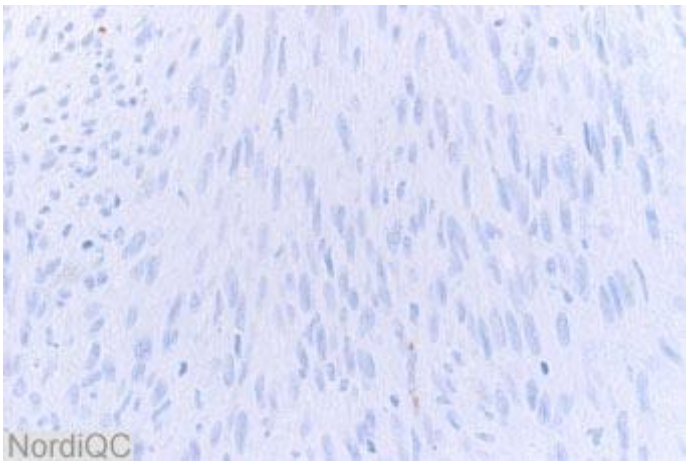

Fig. 2b
Insufficient staining Melan A of the desmoplastic melanoma (same field as in Fig 2a). All the neoplastic cells are virtually negative (same protocol used in Fig. 1b).

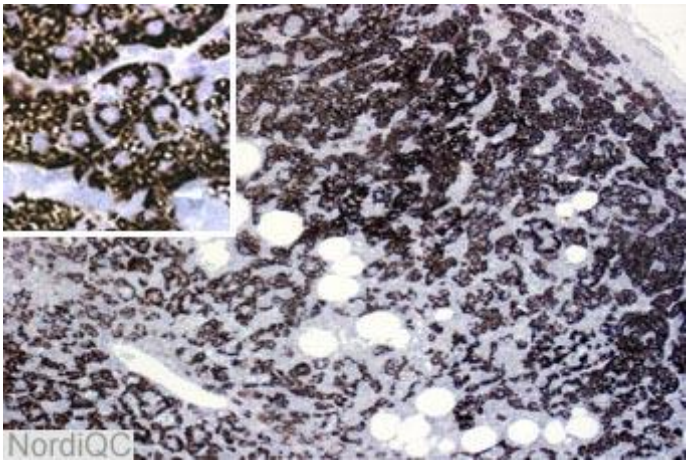

Fig. 3a
Optimal staining for Melan A (clone A103) of the adrenal gland. All the epithelial cells, including zona reticularis demonstrated (same protocol used in Fig. 1a and 2a).

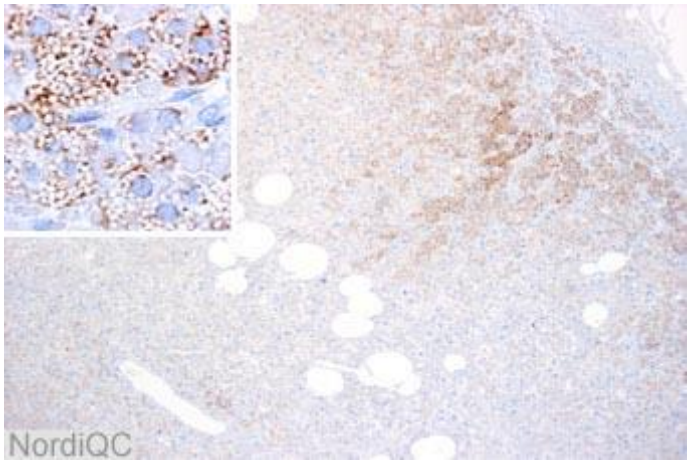

Fig. 3b
Staining for Melan A (clone A103) of the adrenal gland using an insufficient protocol (same protocol used in Fig. 1b and 2b). Only the epithelial cells in zona glomerulosa show a moderate positive staining, while zona reticularis is negative (same field as in Fig. 3a).

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