Assessment Run 20 2007

Melan A (MLA)

The slide to be stained for MLA comprised:
1. Skin, 2. Adrenal gland, 3. Desmoplastic malignant melanoma, 4. Granulosa cell tumour (ovary), 5. Metastatic malignant melanoma.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MLA staining as optimal included:

- A strong, distinct cytoplasmic staining of the normal melanocytes in the skin.
- A strong, distinct cytoplasmic staining of the majority of the neoplastic cells of the metastatic malignant melanoma .
- A moderate, distinct cytoplasmic staining of scattered cells in the desmoplastic malignant melanoma.
- A strong, distinct granular cytoplasmic staining in virtually all the adrenal cortical cells (clone A103).
- A moderate to strong, distinct cytoplasmic staining of the luteinized cells in the granulosa cell tumour.

90 laboratories submitted stains. At the assessment 16 achieved optimal marks (18 %), 27 good (30 %), 28 borderline (31 %) and 19 poor marks (21 %).

The following Abs were used:
mAb clone **A103** (Dako, n=66; Novocastra, n=7; Ventana, n=6; NeoMarkers, n=4; Monosan, n=1)
mAb clones **A103 + M2-7C10 + M2-9E3** (Zymed, n=1)
mAb clones **M2-7C10 + M2-9E3** (NeoMarkers, n=2; BioCare, n=1)
mAb clones **M2-7C10 + M2-9E3 + HMB45 + T311** (BioCare, n=1; Zytomed, n=1)

Optimal staining for MLA in this assessment was obtained with the mAb clone **A103** (15 out of 84) and mAb cocktail **A103+M2-7C10+M2-9E3** (1 out of 1).

Using the mAb clone **A103** the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 or PTM buffer 4 pH 9.0 (Labvision) as buffer. The mAb was typically diluted in the range of 1:20 – 1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 41 out of 58 (71 %) laboratories produced a sufficient (optimal or good) staining. All of 7 protocols based on the use of **A103** as a Ready-To-Use (RTU) Ab gave an insufficient staining result (weak/false negative), in spite of protocol settings being otherwise identical to those based on a concentrated Ab giving sufficient results.

Using the mAb cocktail of the clones **A103 + M2-7C10 + M2-9E3** the protocol giving an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 as HIER buffer. The Ab was diluted 1:100.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- HIER in non-alkaline buffer (typically Citrate pH 6.0)
- Insufficient HIER (too short heating time)
- Less successful primary antibody
- Less successful ready-to-use (RTU) mAb clone **A103**

Almost all laboratories were able to detect MLA in the normal melanocytes in the skin and in the metastatic malignant melanoma, whereas the insufficient staining was characterized by a too weak or entirely negative reaction of MLA in the desmoplastic melanoma and melanoma and the granulosa cell tumour (when using clone A103).
Using clone **A103** (single or in a cocktail) the insufficient staining of the desmoplastic melanoma typically was accompanied by a too weak or false negative reaction of the granulosa cell tumour in which especially the lutenized cells were difficult to demonstrate, unless a high sensitive protocol was applied.

With clone **A103** adrenal gland is an appropriate control for MLA. Virtually all the epithelial cells throughout the adrenal cortex should show a strong cytoplasmic reaction (while medullar cells are unstained). Using other clones such as **M2-7C10** (which does not react with steroid producing cells) normal skin should serve as control: The staining reaction in melanocytes should be as strong as possible without any background reaction.

In the assessment of MLA run 16, 79 laboratories participated out of which 54 (68%) obtained an insufficient

mark. Each was given a specific recommendation to improve their protocol. 40 of them submitted a new MLA stain in run 20. 22 followed the recommendation, of which 17 improved to good or optimal (77 %). 18 laboratories did not follow the recommendation, and 2 of these (11 %) obtained a sufficient staining in run 20.

Compared to run 16 the proportion of sufficient results has been increased from 32% to 48%.

Conclusion
The mAb clone **A103** used single or in a cocktail with **M2-7C10 + M2-9E3** 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. Normal adrenal glad is appropriate for control: The Ab concentration has to be calibrated to give the strongest possible reaction without cross reaction. Clone **A103** as a Ready-To-Use reagent cannot be recommended.
Specific recommendations given to the participating laboratories seem to have an impact on the performance. However the number of insufficient results still is high.

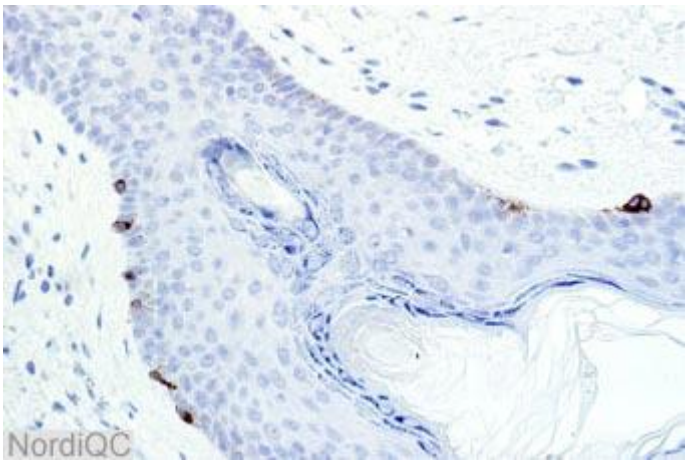

Fig. 1a
Optimal staining for Melan A of the skin. The normal melanocytes show a strong cytoplasmic staining with no reaction of the squamous epithelial cells.

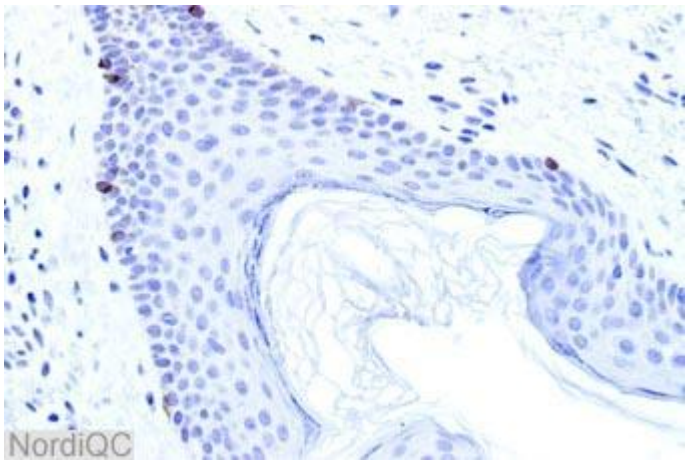

Fig. 1b
Staining for Melan A of the skin using an insufficient protocol (same field as in Fig. 1a.). The melanocytes are demonstrated. However, compare with Fig. 2b - 4b, same protocol. Normal melanocytes can thus not be recommended as control for Melan A.

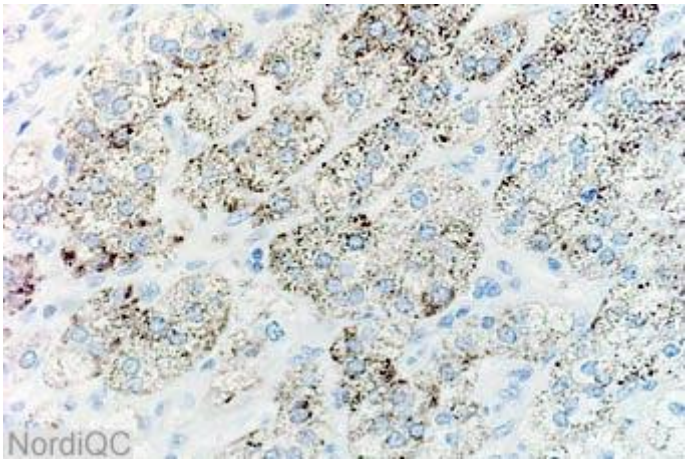

Fig. 2a
Optimal staining for Melan A (clone A103) of the adrenal gland. The majority of the epithelial cells in zona reticularis show a distinct, granular cytoplasmic reaction (same protocol used in Fig. 1a - 4a).

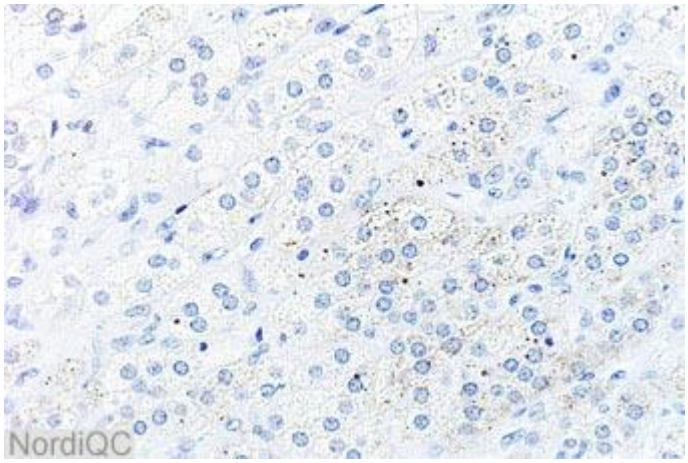

Fig. 2b
Staining for Melan A (clone A103) of the adrenal gland using an insufficient protocol (same protocol used in Fig. 1b - 4b. Only scattered epithelial cells in zona reticularis show a weak reaction (same field as in Fig. 2a).

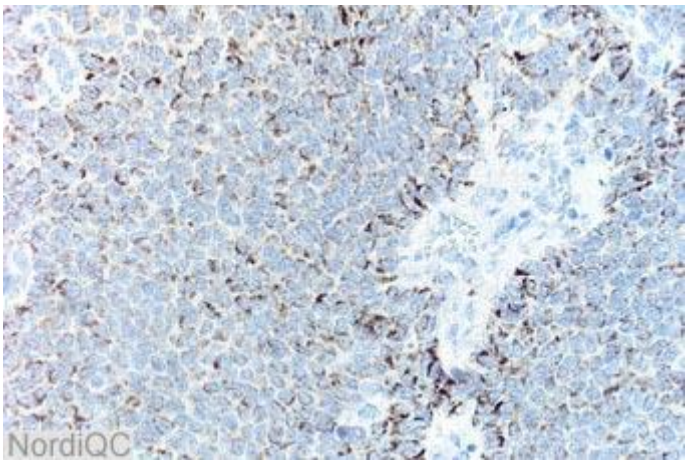

Fig. 3a
Optimal staining for Melan A of the granulosa cell tumour. The majority of the neoplastic cells show a moderate cytoplasmic staining (same protocol used in Fig. 1a - 4a).

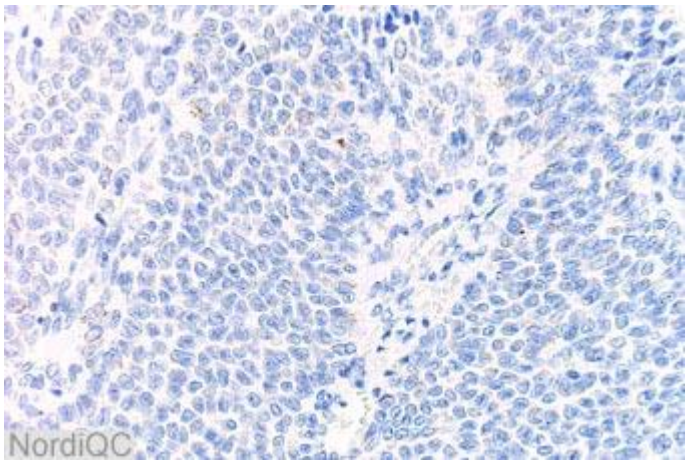

Fig. 3b
Insufficient staining for Melan A of the granulosa cell tumour (same field as in Fig 3a). The neoplastic cells are virtually negative (same protocol used in Fig. 1b - 4b).

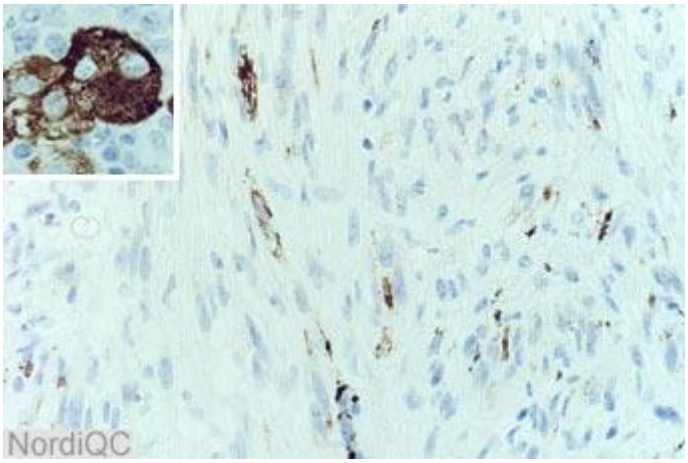

Fig. 4a
Optimal staining for Melan A of the desmoplastic melanoma. Focally the neoplastic cells show a distinct, cytoplasmic dot-like reaction (same protocol used in Fig. 1a – 4a). Insert same protocol in the metastatic melanoma showing an intense reaction

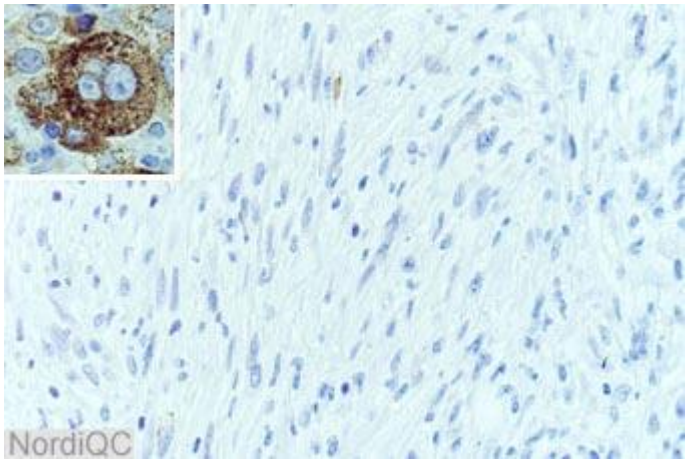

Fig. 4b
Insufficient staining for Melan A of the desmoplastic melanoma (same field as in Fig 4a). All the neoplastic cells are virtually negative (same protocol used in Fig. 1b – 4b). Insert same protocol in the metastatic melanoma showing a strong reaction, stressing the importance to calibrate the protocol on low antigen expressing tumors/cells.

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