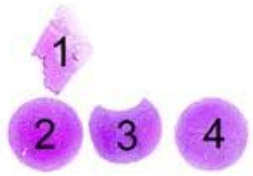
Assessment Run 20 2007

Melanoma Specifik Antigen (MSA)

The slide to be stained for MSA comprised:
1. Skin, 2. Kidney, 3-4. Malignant melanomas.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a MSA staining as optimal included:

- A strong, distinct cytoplasmic staining of virtually all the neoplastic cells of the malignant melanoma no. 3 and ≥ 50 % of the neoplastic cells of the malignant melanoma no. 4.
- No reaction in the kidney.
- No reaction in the skin with the exception of scattered reactive melanocytes.

102 laboratories submitted stains. Of these 3 used an inappropriate antibody. Assessment of the remaining 99 laboratories gave following results: 50 achieved optimal marks (51 %), 46 good (46 %), and 3 borderline (3 %). None of the laboratories got poor marks.

The clone **HMB-45** was used of all the laboratories (Dako, n=85; NeoMarkers, n=4; Enzo, n=3; BioGenex, n=2; Novocastra, n=2; Ventana, n=2; Cell Marque, n=1).

An optimal staining for MSA with clone HMB-45 could in this assessment both be obtained without pre-treatment (5 out of 8 labs) or by using HIER (heat induced epitope retrieval) as pre-treatment (45 out of 87).

The HIER buffers used were: Tris-EDTA/EGTA pH 9 (27/48)*, Cell Conditioning1 (Benchmark, Ventana) (4/16), Citrate pH 6 (6/9), Target retrieval Solution pH 6.1 (Dako) (3/3), EDTA pH 8 (1/3), Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) (2/2), 1mM EDTA, pH 9.0 (1/1).
* (number of optimal results/number of laboratories using this buffer)

The mAb was typically diluted in the range of 1:25 – 1:400 depending on the total sensitivity of the protocol employed or as a Ready-To-Use antibody. Using these settings 91 out of 93 (98 %) laboratories produced a sufficient staining (optimal or good). Proteolytic pre-treatment could not be used to obtain an optimal staining and 2 out of 4 were insufficient.

The causes of insufficient staining were:
- Too low concentration of the primary antibody
- Proteolytic pre-treatment

96 out of 99 laboratories obtained a sufficient result. The multitissue block consisted of two metastatic melanomas with different MSA expression. The insufficient results were all characterized by a too weak staining reaction in one of the melanomas.
Both HIER and omission of pre-treatment could in this assessment be used to obtain an optimal staining and no major difference was seen in the pass rate comparing these two protocol settings. However, in the previous run 7 2003, the performance without HIER was lower, and from a general point of view HIER should be included in a standard protocol setting for MSA to reduce any masking of the antigen by prolonged formalin fixation. Omission of HIER may, however, be beneficial in cases of problems with tissue detachment. Both procedures should be validated by the individual laboratories in order to obtain the same sensitivity in the two protocols (i.e., different concentrations of the primary Ab may be necessary).

The proportion of sufficient MSA stains has increased from 74% in run 7 to 97% in the current run, indicating that with a proper adjustment of the protocol, clone HMB-45 is a robust Ab.

As normal melanocytes do not express MSA, melanocytic tumour tissue is needed for control . The staining of the tumour cells must be as strong as possible without staining in other cells. A multitissue block combining junction/compound nevi and malignant melanomas with varying expression of MSA should be considered.

Conclusion
The mAb clone HMB45 is a robust Ab for the detection of MSA. HIER is recommended for pretreatment even though omission of pre-treatment also gives good results.

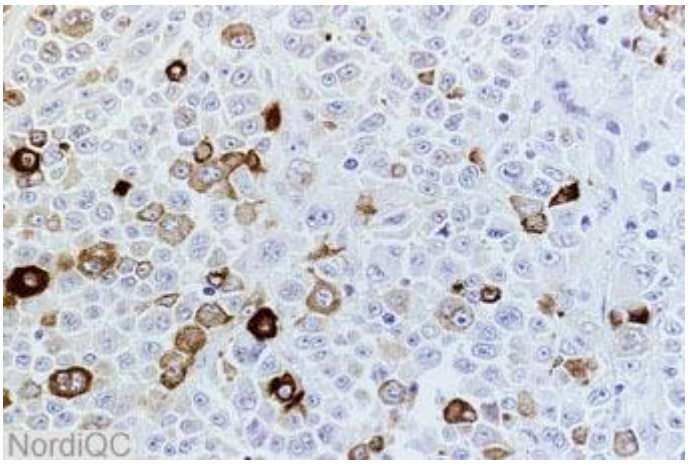


Fig. 1a
Optimal staining for MSA of the malignant melanoma no. 4. A strong, distinct staining reaction is seen in a large proportion of the neoplastic cells.

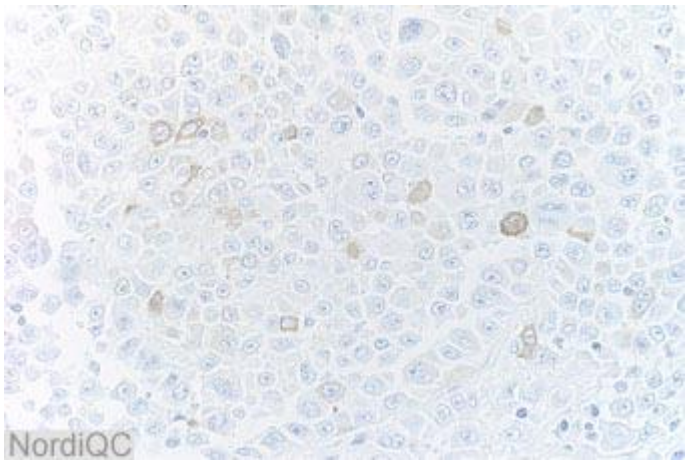


Fig. 1b
Staining for MSA of the malignant melanoma no. 4 using an insufficient protocol (same field as in fig 1a). Both the proportion of positive cells and the staining intensity is reduced compared to Fig. 1 a., due to a too low concentration of HMB45 applied.

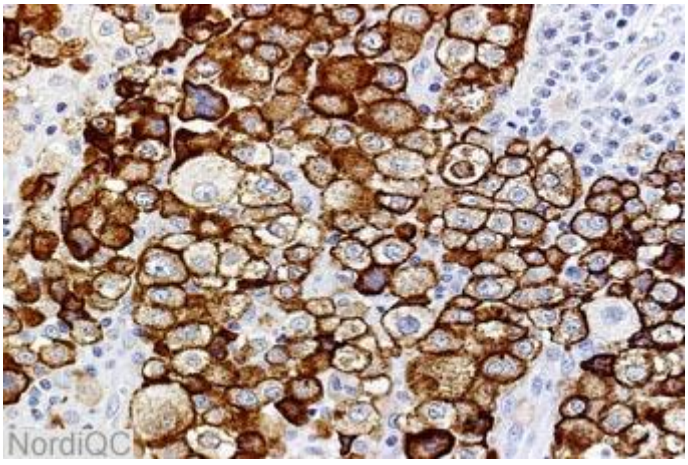


Fig. 2a
Optimal staining for MSA of the malignant melanoma no. 3. A strong, distinct staining reaction is seen in a large proportion of the neoplastic cells. The reaction is mainly localized at the membranes of the neoplastic cells but also a granular cytoplasmic reaction is seen. The protocol was based on HIER.

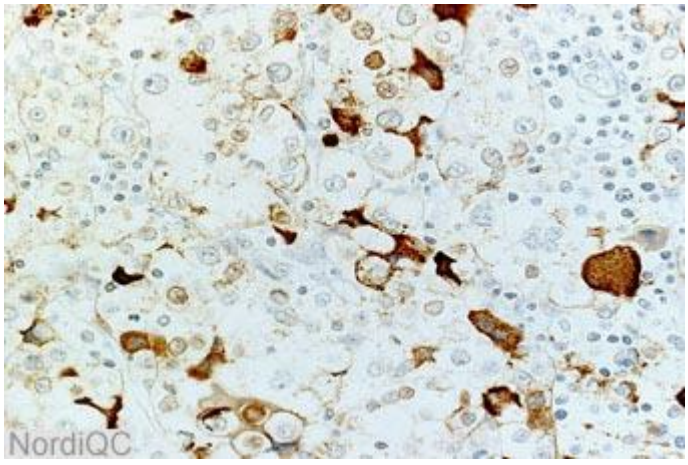


Fig. 2b
Staining for MSA of the malignant melanoma no. 3 using an insufficient protocol based on proteolytic pre-treatment. The proportion of positive cells is reduced compared to Fig. 2a (same field), and the morphology is impaired due to the excessive digestion causing extraction of the cytoplasmic epitope.

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