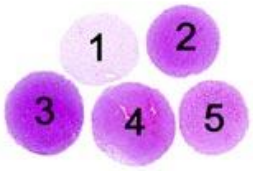
## Assessment Run 24 2008

# Melan A (MLA)

The slide to be stained for Melan A (MLA) comprised:
1. Angiomyolipoma, 2. Malignant melanoma, 3, Granulosa cell tumour, 4, Adrenal gland, 5. Kidney.
All tissues were fixed in 10% neutral buffered formalin.




Criteria for assessing a MLA staining as optimal included:

- A strong, distinct granular cytoplasmic staining in virtually all the adrenal cortical cells (clone A103).
- A strong, distinct cytoplasmic staining of the majority of the neoplastic cells of the malignant melanoma.
- A moderate to strong, distinct cytoplasmic staining of the neoplastic cells of the angiomyolipoma and the luteinized cells in the granulosa cell tumour.
- No or only minimal staining of the kidney.

115 laboratories submitted stains. At the assessment 24 achieved optimal marks (21%), 34 good (29%), 38 borderline (33 %) and 19 poor marks (17 %).

The following Abs were used:
mAb clone **A103** (Dako, n=78; Ventana, n=11; Novocastra/Leica, n=9; NeoMarkers/Thermo, n=5; Monosan, n=1)
mAb clones **A103 + M2-7C10 + M2-9E3** (Zymed, n=2)
mAb clones **M2-7C10 + M2-9E3** (NeoMarkers/Thermo, n=2; BioCare, n=1)
mAb clones **M2-7C10 + M2-9E3 + T311** (BioCare, n=1)
mAb clones **HMB45 + M2-7C10 + M2-9E3 + T311** (BioCare, n=3; Zytomed, n=1)
pAb **RB-9054** (Neomarkers/Thermo, n=1)

Optimal staining for MLA in this assessment was only obtained with the mAb **A103** (24 out of 104).

All optimal protocols were based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9.0 (17/42)*, Target Retrieval Buffer pH 9, (Dako) (4/16), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/3) or Citrate pH 6.0 (1/12) as HIER buffer. The mAb was diluted in the range of 1:10 – 1:100 depending on the total sensitivity of the protocol employed or as a Ready-To-Use Ab (Dako IR633).
Using these protocol settings 44 out of 58 (76 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient HIER (too short HIER time and/or a non-alkaline buffer)
- Less successful ready-to-use (RTU) mAb clone **A103**
- False negative staining combined with a false positive staining of endogenous biotin

In this assessment the prevalent feature of an insufficient staining was a false negative reaction of the granulosa cell tumour and the angiomyolipoma. In general, almost all laboratories were able to detect MLA in the malignant melanoma. This observation was in concordance with previous assessment of MLA and stresses that a highly sensitive IHC system is mandatory to demonstrate MLA in tumours with low MLA expression as granulosa cell tumours, desmoplastic melanoma etc.
An applicable critical staining 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. However this reaction pattern can only be applied when a non-biotin based detection system is used, as the adrenal cortical cells are rich on endogenous biotin and a false positive reaction will thus mimic the specific reaction and eliminate the potential as a reliable control.

In the last 3 assessments it has been shown that the most successful clone for MLA is **A103** and the protocol settings and Ab. format are important parameters to obtain a sufficient result. From the 3 assessments virtually

only an alkaline buffer as Tris-EDTA/EGTA pH 9 or similar and a relative high conc. of the primary Ab. ranging from 1:10 – 100 could be used to obtain a sufficient result, while protocols based on other HIER buffers and a reduced concentration of the Ab. typically gave an insufficient result. Also the RTU formats of the clone A103 have shown a variable performance, which seems to be related to the sensitivity of the total RTU system and the calibration of this.

Table 1 shows the cumulated data of the performance and protocol settings of the clone A103 in the latest three MLA assessments.

Table 1. **Cumulated data for clone A103 in three runs**

| | **Run 16, 20 & 24 All protocol settings** | | | **Run 16, 20 & 24 Optimal protocol settings*** | | |
|---|---|---|---|---|---|---|
| | **Protocols** | **Sufficient** | **Optimal** | **Protocols** | **Sufficient** | **Optimal** |
| mAb clone A103 conc. | 239 | 112 (47%) | 47 (20%) | 139 | 106 (76%) | 47 (34%) |
| mAb clone A103 RTU VMS | 23 | 3 (13%) | 0 (0%) | 0 | 0 (0%) | 0 (0%) |
| mAb clone A103 RTU Dako | 4 | 4 (100%) | 2 (50%) | 2 | 2 (100%) | 2 (100%) |

*HIER in an alkaline buffer such as Tris-EDTA/EGTA pH 9 or equivalent and an appropriate dilution of the antibody listed in the three assessments.

This was the 4th assessment of MLA and as shown in table 2 the pass rate and proportion of sufficient stainings has been slightly improved in the last 3 runs, but is still on a low level.

Table 2. **Pass rate in four runs**

| | Run 7 2003 | Run 16 2006 | Run 20 2007 | Run 24 2008 |
|---|---|---|---|---|
| Participants, n= | 35 | 79 | 90 | 115 |
| Sufficient results | 69% | 32% | 48% | 50% |

In the previous assessment of MLA (run 20), 90 laboratories participated out of which 47 (52%) obtained an insufficient mark. Each was given a specific recommendation to improve their protocol. 40 of them submitted a new MLA stain in run 24. 18 followed the recommendation, of which 10 improved to good or optimal (56 %). 17 laboratories did not follow the recommendation, and 1 of these (6 %) obtained a sufficient staining in run 24. 5 laboratories changed their entire IHC system and 3 of these obtained a sufficient staining.

In total 112 laboratories obtaining an insufficient result in run 7, 16 and run 20 have been given specific recommendations how to improve their protocol. 89 laboratories of them submitted a new MLA stain in the subsequent run. 44 followed the recommendation, of which 28 improved to good or optimal (64 %). 40 laboratories did not follow the recommendation, and 3 of these (9 %) obtained a sufficient staining in the subsequent run.

**Conclusion**

The mAb clone **A103** seems be to the most sensitive and robust marker for MLA. HIER in an alkaline buffer such as Tris-EDTA/EGTA pH 9 is highly recommended for optimal performance. Normal adrenal gland is an appropriate control: Virtually all the cortical epithelial cells must show a strong distinct granular staining. Biotin based detection systems can not be recommended for MLA due to the risk of false positive reaction due to endogenous biotin.

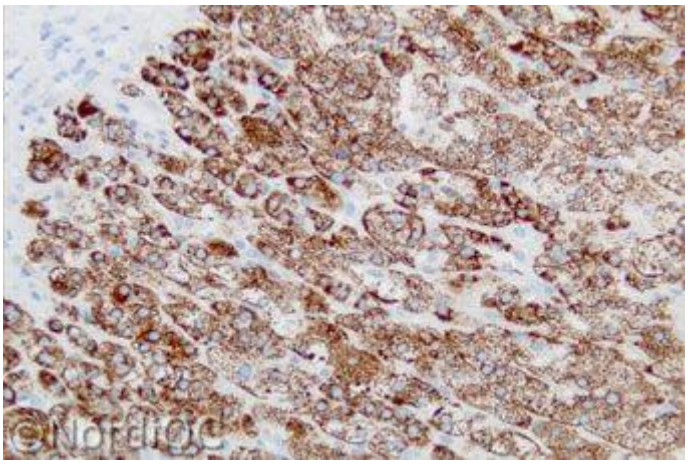



Fig. 1a
Optimal MLA staining of the adrenal gland using the clone A103 optimally calibrated and with HIER in an alkaline buffer. The majority of the epithelial cells in zona reticularis show a distinct, granular cytoplasmic reaction.

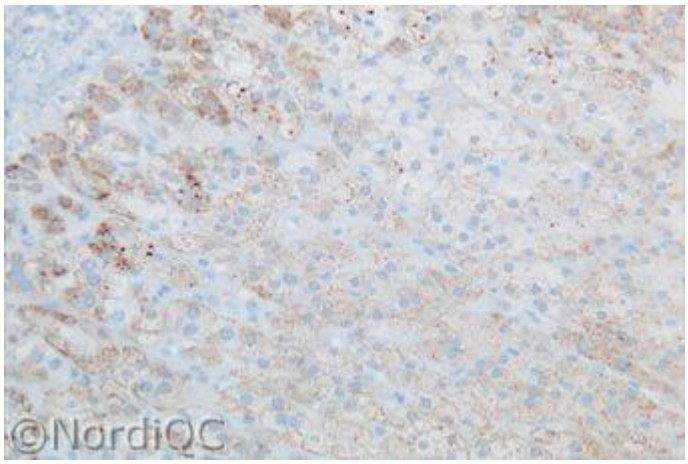



Fig. 1b
MLA staining of the adrenal gland (same field as in Fig. 1a.) using an insufficient protocol based on the clone A103 but too diluted. Only scattered epithelial cells in zona reticularis show a weak reaction. Also compare Fig. 3b, same protocol.

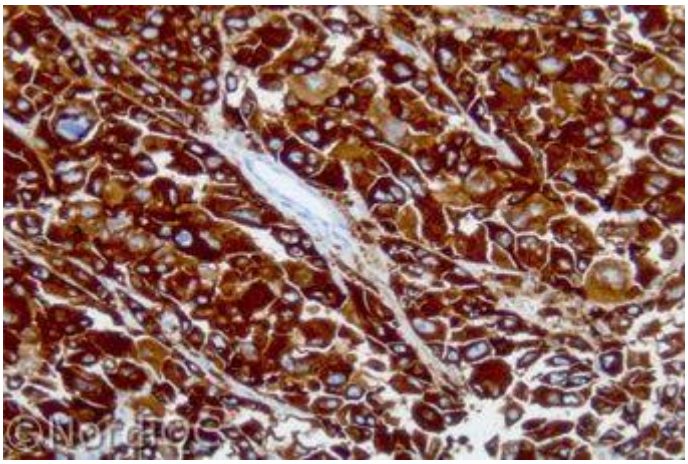


Fig. 2a
Optimal MLA staining of the melanoma using same protocol as in Fig. 1a. All the neoplastic cells show an intense staining, but still no background reaction is seen.

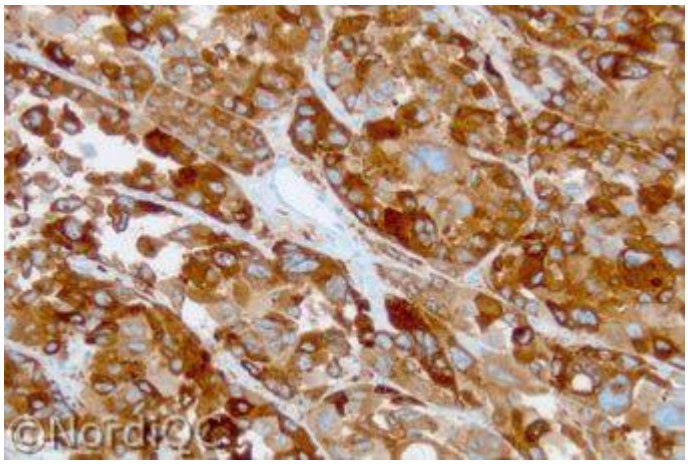



Fig. 2b
MLA staining of the melanoma using same protocol as in Fig. 1b. All the neoplastic cells are demonstrated. However these cells express a high concentration of MLA and can not be used to monitor the sensitivity of the protocol. Also compare with Fig. 3b, same protocol

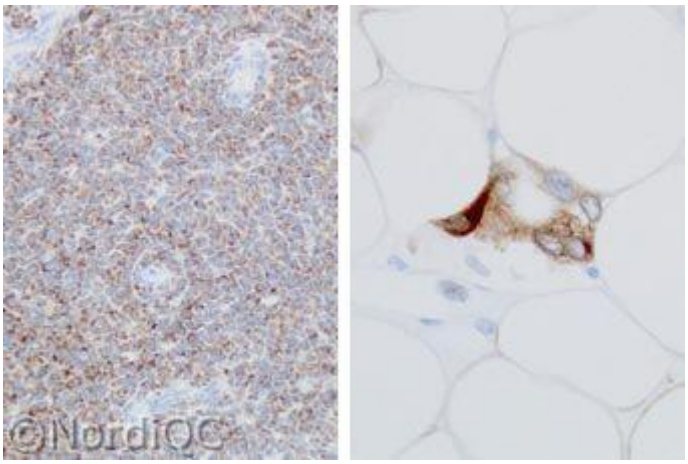



Fig. 3a
Optimal MLA staining using same protocol as in Figs. 1a & 2a.
Left: Granulosa cell tumour: The majority of the neoplastic cells show a moderate cytoplasmic staining
Right: Angiomyolipoma: Scattered neoplastic cells show a moderate to strong cytoplasmic staining.

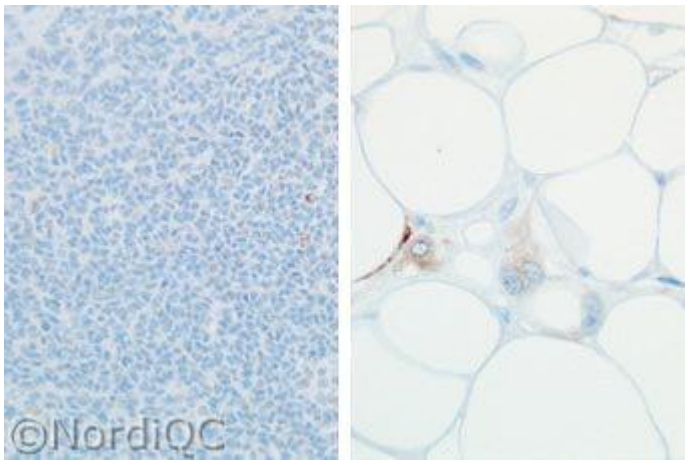



Fig. 3b
Insufficient MLA staining using same protocol as in Figs. 1b & 2b. (same field as in Figs. 3a).
Left: Granulosa cell tumour: The neoplastic cells are virtually negative.
Right: Angiomyolipoma: The neoplastic cells only show a weak and dubious staining.

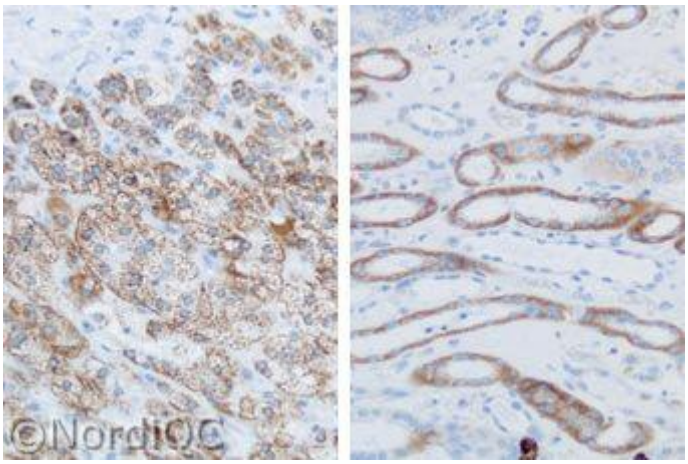



Fig. 4a
Insufficient MLA staining based on the clone A103 (too diluted) with HIER in an alkaline buffer and a biotin based detection system.
Left: Adrenal gland. The epithelial cells in zona reticularis show a distinct, granular cytoplasmic reaction. However this is caused by endogenous biotin and the sensitivity can therefore not be evaluated. Also see Fig. 4b.
Right: Kidney. A distinct granular reaction is seen in the tubular epithelial cells due to endogenous biotin.

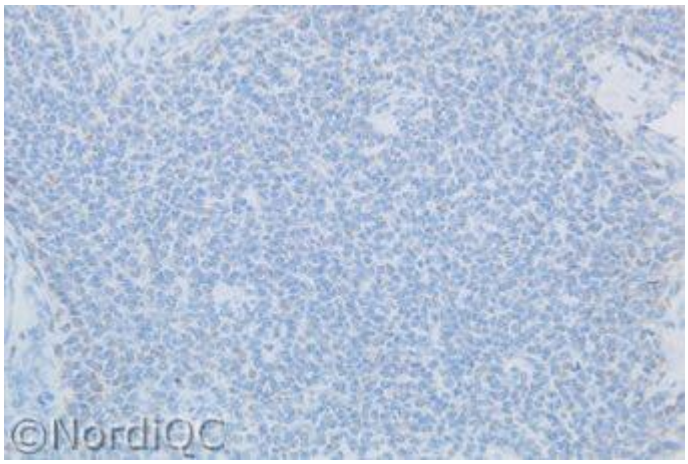



Fig. 4b
Insufficient MLA staining of the granulosa cell tumour using same protocol as in Fig. 4a. The neoplastic cells are virtually negative.

SN/HN/MV/LE 8-12-2008