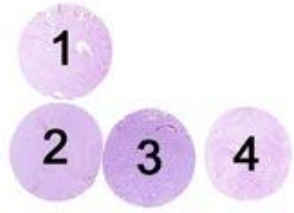
Assessment Run 31 2011

Melan A (MLA)

The slide to be stained for MLA comprised:

1. Kidney, 2. Malignant melanoma, 3. Ovarian granulosa cell tumour, 4. Adrenal gland.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MLA staining as optimal included:

- A moderate to strong, distinct granular cytoplasmic staining in virtually all the adrenal cortical cells when using clone A103, otherwise the adrenal gland should be unstained.
- A moderate to strong, distinct cytoplasmic staining of the majority of the neoplastic cells of the malignant melanoma.
- An at least weak to moderate granular cytoplasmic staining of the majority of the neoplastic cells of the granulosa cell tumour when using clone A103, otherwise the tumour should be unstained.
- No or only a minimal staining of the kidney.

166 laboratories participated in this assessment. 1 lab used an inappropriate antibody (HMB45). Of the remaining 165 laboratories 66 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for MLA, run 31**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A103**	78 12 8 2 1	Dako Leica/Novocastra NeoMarkers Monosan Cell Marque	24	36	31	10	60 %[3)]	64 %[3)]
mAb clone **MC-7C10**	2 1	Zytomed Cell Marque0	0	2	1	0	-	-
mAb clone cocktail **MC-7C10+M2-9E3**	3 2 1 1	NeoMarkers Biocare DBS Master Diagnostica	0	4	3	0	57 %	-
mAb clone cocktail **HMB45+MC-7310 +M2-9E3+T311**	3 1	Biocare DBS	2	2	0	0	-	-
mAb clone cocktail **A103+MC-7C10 +M2-9E3**	2	Zymed/Invitrogen	0	0	1	1	-	-
rmAb clone **A19-P**	1	DB Biotech	0	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **A103, IR633**	21	Dako	13	7	1	0	95 %	100 %
mAb clone **A103, 790-2990**	21	Ventana	0	15	6	0	71 %	-
mAb clone **A103, PA0233**	3	Leica/Novocastra	1	1	1	0	-	-
mAb clone **M2-7C10, 281M-97**	1	Cell Marque	0	1	0	0	-	-
mAb clone cocktail **M2-7C10 + M2-9E3 PM077**	1	Biocare	0	1	0	0	-	-
Total	165		40	70	44	11	-	

Proportion			24 %	42 %	27 %	7 %	66 %	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) The results are highly dependent of the staining platform, se below.

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **A103**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (6/20)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (11/17), Bond Epitope Retrieval Solution 2 (Bond, Leica) (5/14), Cell Conditioning 1 (BenchMark, Ventana)(1/32) or EDTA/EGTA pH8 (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:25– 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 46 out of 72 (84 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone cocktail **HMB45 + MC-7C10+M2-9E3 + T311**: The protocols giving an optimal result were based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (1/1) as the retrieval buffer. The mAb was diluted in the range of 1:100 – 1:400. Using these protocol settings both of 2 (100 %) laboratories produced an optimal staining.

Ready-To-Use Abs
mAb clone **A103** (prod. no. IR633, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1) and an incubation time of 20-30 min in the primary Ab and EnVision Flex (K8000) or Envision Flex+ (K8002) as the detection system. Using these protocol settings all of 19 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **A103** (product.no. PA0233, Leica/Novocastra): The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica), an incubation time of 20 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system. Using this protocol setting both of 2 laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Insufficient HIER (use of an non-alkaline buffer and/or too short efficient HIER time)
- Usage of detection systems with too low sensitivity
- False positive staining of endogenous biotin.

In this assessment and in concordance with the previous NordiQC assessments for MLA the prevalent feature of an insufficient staining was a false negative reaction of the cells and structures expected to be demonstrated. Almost all laboratories were able to detect MLA in the malignant melanoma. For the laboratories using the mAb clone A103 either as single Ab or in a cocktail with other clones, the demonstration of MLA in the granulosa cell tumour was much more challenging and required an optimal protocol based on a correct titre of the primary Ab, efficient HIER in an alkaline buffer and a sensitive IHC detection system.

A significant difference in the overall performance for MLA mAb clone A103 seemed to be highly related to the IHC platform applied: Only 6 out of 50 (12 %) protocols based on mAb clone A103 as a concentrate and performed on the fully automated IHC platform BenchMark XT or Ultra (Ventana) were assessed as sufficient, and only 1 was assessed as optimal (2 %). In contrast, 12 out of 16 (75 %) protocols based on the same Ab and similar protocol settings (titre range and HIER in an alkaline buffer) performed on a comparable fully automated platform Bond-Max or Bond III (Leica) were assessed as sufficient, out of which 6 (43 %) were optimal. The difference most likely is related to the sensitivity level provided by the two systems for the mAb clone A103. The optimal protocol performed on the BenchMark Ultra was based on a titre of 1:25 of the primary Ab (Dako) and 32 min. incubation, compared to a titre range of 1:50-200 using same Ab and incubation time on the Bond platform.
The most successful and robust assay for MLA in this assessment was obtained by the Ready-To-Use system based on the mAb clone A103 from Dako giving a pass rate of 95 % (20 out of 21 laboratories) out of which 62 % were assessed as optimal. In comparison the Ready-To-Use system from Ventana based on the same mAb gave a pass rate of 71 % (15 out of 21 laboratories), but none were assessed as optimal.

With the clone A103 an applicable critical staining quality indicator was the adrenal cortex: A moderate to strong granular cytoplasmic reaction in virtually all the epithelial cells throughout the gland must be seen. However, this reaction pattern can only be identified when a non-biotin based detection system is used, as the adrenal cortical

cells are rich in endogenous biotin. Hence, a false positive reaction will mimic the specific reaction and eliminate the adrenal cortex as a reliable control. In this assessment 7 out of the 55 insufficient results showed both a false negative staining and a false positive staining due to endogenous biotin.

Only few labs used other MLA clones than A103, most often in a cocktail with clone HMB45. The staining results do not allow any firm conclusion as regards the Ab and protocol settings.

This was the 5th assessment of MLA in NordiQC. The proportion of sufficient results continues to increase despite many new participants. However the pass rate is still relatively low as shown in table 2:

Table 2. **Proportion of sufficient results for MLA in the five NordiQC runs performed**

	Run 7 2003	Run 16 2006	Run 20 2007	Run 24 2008	Run 31 2011
Participants, n=	35	79	90	115	165
Sufficient results	69 %	32 %	48 %	50 %	66 %

Conclusion

The mAb clone A103 is the most widely used clone for MLA and is highly recommendable.
Efficient HIER in an alkaline buffer in combination with a sensitive and specific IHC system is mandatory for optimal performance. In this assessment the Ready-To-Use system for MLA from Dako gave far the highest proportion of sufficient results. 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.

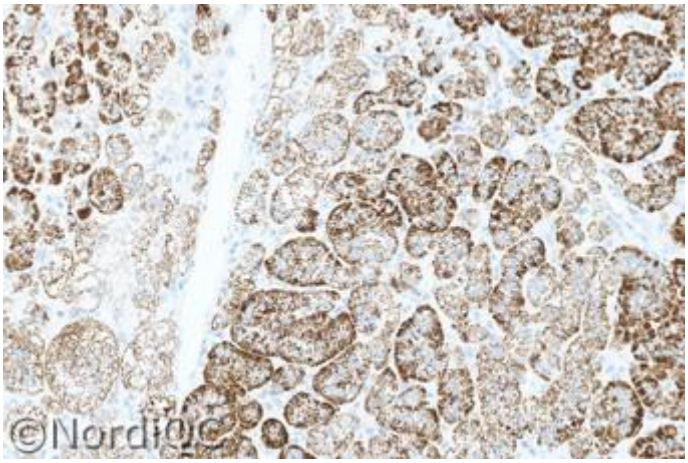


Fig. 1a
Optimal MLA staining of the adrenal gland using the mAb clone A103 carefully calibrated and applied with HIER in an alkaline buffer. The majority of the epithelial show a distinct, granular cytoplasmic staining reaction. No background reaction is seen. Also compare with Figs. 2a – 4a, same protocol.

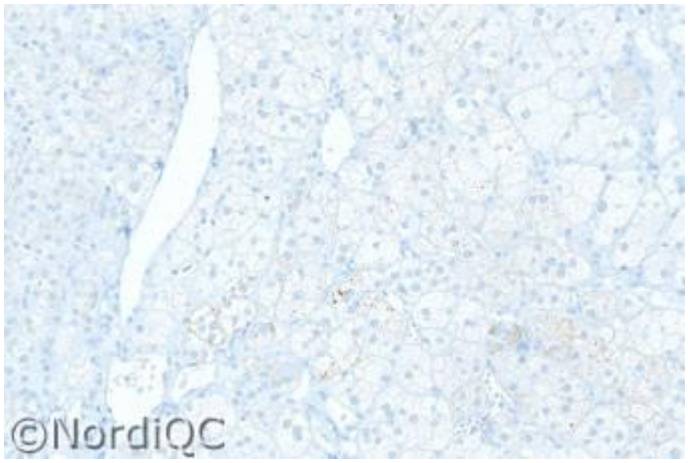


Fig. 1b
Insufficient MLA staining of the adrenal gland - same field as in Fig. 1a. using the mAb clone A103 too diluted. Only scattered epithelial cells show a weak staining reaction. Also compare Figs. 2b & 3b, same protocol.

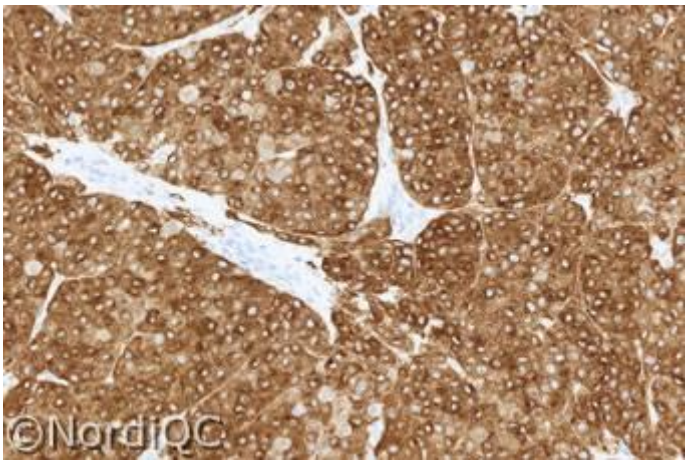


Fig. 2a
Optimal MLA staining of the malignant melanoma using same protocol as in Fig. 1a. All the neoplastic cells show a strong cytoplasmic staining reaction. No background reaction is seen.

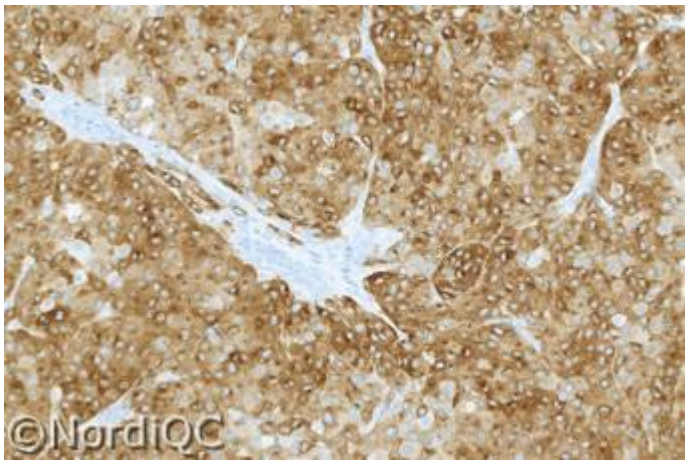


Fig. 2b
MLA staining of the melanoma using same protocol as in Fig. 1b. – same field as in Fig. 2b. All the neoplastic cells are demonstrated. However, compare with Fig. 3b, same protocol.

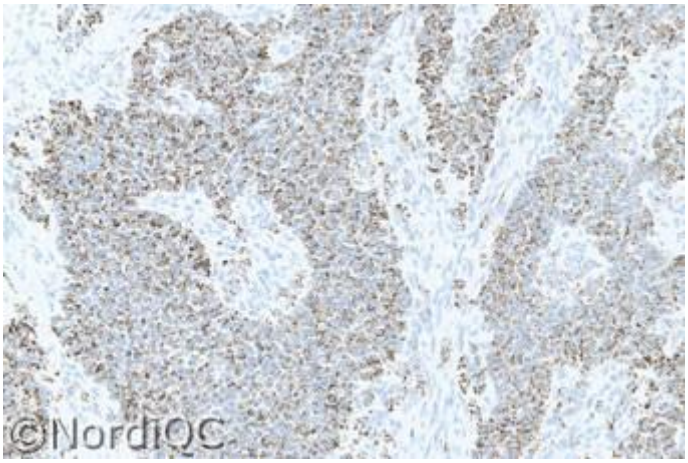


Fig. 3a
Optimal MLA staining of the granulosa cell tumour using same protocol as in Figs. 1a & 2a. The majority of the neoplastic cells show a weak to moderate granular cytoplasmic staining reaction.

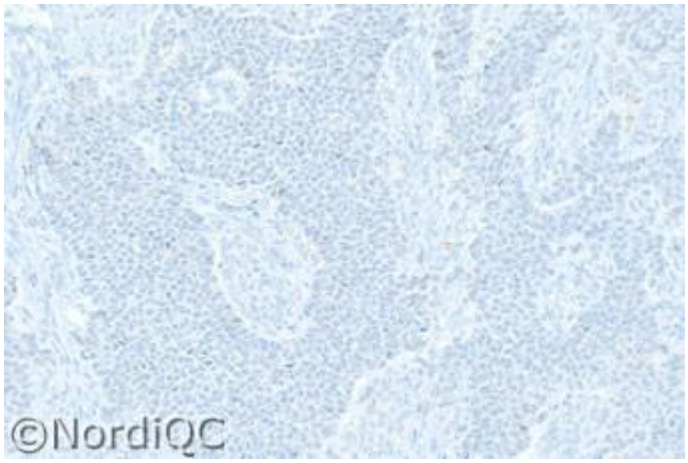


Fig. 3b
Insufficient MLA staining of the granulosa cell tumour using same protocol as in Figs. 1b & 2b. - same field as in Fig. 3a. Only scattered neoplastic cells only show a weak or equivocal staining reaction.

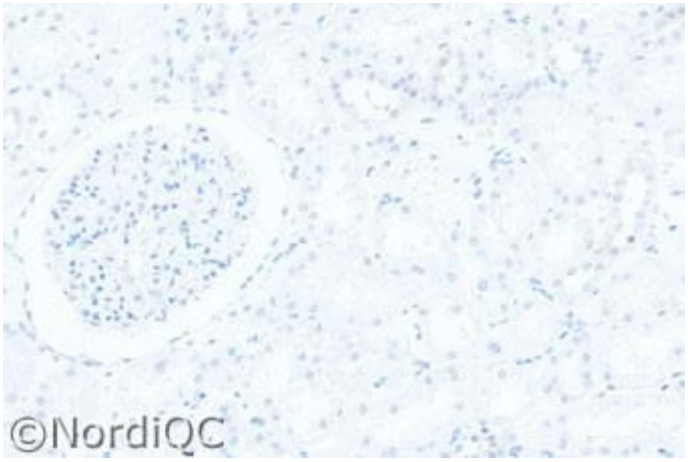


Fig. 4a
Optimal MLA staining of the kidney using same protocol as in Figs. 1a – 3a. No staining reaction is seen in the epithelial cells.

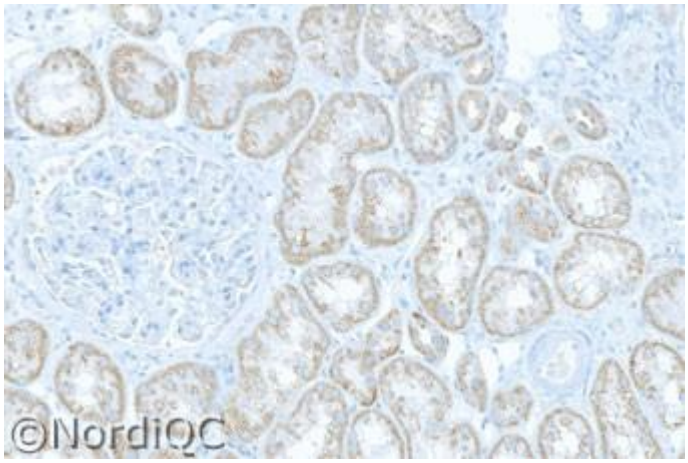


Fig. 4b
Insufficient MLA staining of the kidney. Usage of efficient HIER in an alkaline buffer in combination with a biotin based detection system reveals a false positive granular staining reaction in the epithelial cells due to endogenous biotin.

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