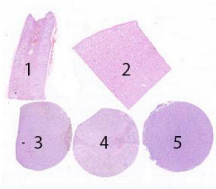
Assessment Run 42 2014
Melan A (MLA)

Material
The slide to be stained for MLA comprised:



1. Adrenal gland, 2. Kidney, 3-4. Malignant melanoma, 5. Ovarian granulosa cell tumour

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 reaction in virtually all adrenal cortical cells (clone A103).
- A moderate to strong, distinct cytoplasmic staining reaction of the vast majority of neoplastic cells in the malignant melanoma tissue core no 3.
- An at least weak to moderate, distinct cytoplasmic staining reaction of the majority of the neoplastic cells in the malignant melanoma tissue core no. 4.
- An at least weak to moderate granular cytoplasmic staining reaction of the majority of the neoplastic cells in the granulosa cell tumour (clone A103).
- No or only a minimal staining in the kidney.

Participation

Number of laboratories registered for MLA, run 42	214
Number of laboratories returning slides	200 (93%)

Results
200 laboratories participated in this assessment. 2 laboratories used an inappropriate antibody (muscle specific antigen). Of the remaining 198 laboratories 68% achieved a sufficient mark. Antibodies used and assessment marks are summarized in Table 1 (see page 2).

The most frequent causes of insufficient staining were:
- Low sensitivity detection systems
- Too short efficient HIER time
- Too low concentration of the primary antibody

Performance history
This was the sixth NordiQC assessment of MLA. Almost identical pass rate to the previous run 31 in 2011 was observed (see Table 2).

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

	Run 7 2003	Run 16 2006	Run 20 2007	Run 24 2008	Run 31 2011	Run 42 2014
Participants, n=	35	79	90	115	165	198
Sufficient results	69%	32%	48%	50%	66%	68%

Conclusion
The mAb clone **A103** was the most frequently used antibody for MLA and is highly recommendable. Efficient HIER, preferable in an alkaline buffer, in combination with a sensitive and specific IHC system were the main prerequisites for optimal performance. Biotin based detection systems cannot be recommended for MLA due to the risk of false positive reaction due to endogenous biotin.
In this assessment, the Ready-To-Use system for MLA from Dako provided the highest proportion of sufficient results and optimal results.
For the mAb clone A103, normal adrenal gland is recommended as positive tissue control. Virtually all cortical epithelial cells must show a strong cytoplasmic granular staining reaction. Kidney is recommended as negative tissue control in which no staining reaction of epithelial cells should be seen.

Table 1. **Antibodies and assessment marks for MLA, run 42**

Concentrated Abs:	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A103**	71 13 1 3 1 4 1 1 5	Dako Leica/Novocastra NeoMarkers Monosan Biogenex Cell Marque Immunologic Genemed Thermo Scientific	33	27	32	8	60%	63%
mAb clone **M2-7C10**	1	Zytomed	1	0	0	0	-	-
mAb clone cocktail **M2-7C10+M2-9E3**	2 1	Master Diagnostica Biocare	2	1	0	0	-	-
mAb clone cocktail **HMB45+MC-7310 +M2-9E3+T311**	3	Biocare	3	0	0	0	-	-
mAb clone cocktail **A103+ M2-7C10+M2-9E3**	1	Novex/life technologies	0	0	1	0	-	-
Ready-To-Use Abs:								
mAb clone **A103, IR633**	43	Dako	32	7	4	0	91%	95%
mAb clone **A103 790-2990**	41	Ventana	7	16	15	3	56%	75%
mAb clone **A103, PA0233**	4	Leica, Novocastra	1	3	0	0	-	-
mAb clone **M2-7C10+M2-9E2**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone cocaktail **M2-7C10 + M2-9E3 PM077**	1	Biocare	0	1	0	0	-	-
Total	198		80	55	52	11	-	
Proportion			40%	28%	27%	5%	68%	

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of MLA, Run 42
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **A103**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (12/21)*, Bond Epitope Retrieval Solution 2 (Bond, Leica) (9/20), Target Retrieval Solution pH 9 (3-in-1) (Dako) (6/10), Cell Conditioning 1 (BenchMark, Ventana)(3/40), Citrate pH 6 (1/4) or Diva pH 6 (Biocare) (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:20–1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 52 of 82 (63%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **M2-7C10**: One protocol with optimal result was based on HIER using Citrate pH 6 as retrieval buffer. The mAb was diluted 1:100 and a 2-step polymer detection kit was used.

mAb clone cocktail **M2-7C10+M2-9E3**: Protocols with optimal results were based on HIER using Cell Conditioning 1 (BenchMark, Ventana) (1/1) or Citrate pH 6 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:50–1:100 depending on the total sensitivity of the protocol employed.

mAb clone cocktail **HMB45 + MC-7C10+M2-9E3 + T311**: Protocols with optimal results were based on HIER using Tris-EDTA/EGTA pH 9 (1/1), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:50–1:600. Using these protocol settings 3 of 3 (100%) laboratories produced an optimal staining.

Table 3. **Proportion of optimal results for MLA for the most commonly used antibody clone A103 as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **A103**	8/16** (50%)	0/1	3/34 (9%)	0/1	8/16 (50%)	0/3

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
mAb clone **A103** (prod. no. IR633, Dako): Protocols with optimal results were all based on HIER in PT-Link using Target Retrieval Solution pH 9 or Target Retrieval Solution pH 9 (3-in-1), 20-30 min. incubation of the primary Ab and EnVision Flex (K8000) or Envision Flex+ (K8002) as detection system. Using these protocol settings 37 of 39 (95%) laboratories produced a sufficient staining (optimal or good).

mAb clone **A103** (product.no. PA0233, Leica/Novocastra): One protocol with optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica), 20 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using this protocol setting 3 of 3 laboratories produced a sufficient staining.

mAb clone **A103** (prod. no. 790-2990, Ventana): Protocols with optimal results were typically based on HIER in Cell Conditioning 1 for 64 min. (BenchMark, Ventana) (7/41), 16-32 min. incubation of the primary Ab using OptiView-DAB (760-700), UltraView-DAB (760-500) or UltraView-RED (760-501) +/- amplification kit as detection system. Using these protocol settings 15 out of 20 (75%) laboratories produced a sufficient staining.

Comments
In this assessment and in concordance with the previous NordiQC assessments for MLA, the prevalent feature of an insufficient staining was a general too weak or completely false negative staining reaction of cells and structures expected to be demonstrated. This was observed in 89% of the insufficient results (56 of 63) and was only seen for protocols based on the mAb clone A103 either on its own or in combination with other clones. In general, almost all laboratories were able to detect MLA in the malignant melanoma, tissue core no. 3, whereas the demonstration of MLA in the malignant melanoma, tissue core no. 4 and the granulosa cell tumour (A103 clone only) was much more challenging and required an optimally calibrated protocol.
A significant difference in the overall performance of the mAb clone A103 seemed to be highly related to the IHC platform used as seen in Table 3. If the Ab was applied on either the Dako Autostainer system or the Leica Bond system, used as a concentrate in the range of 1:20-1:200, HIER in an alkaline buffer, 50% of the laboratories obtained an optimal staining result. In comparison, only 9% of the laboratories using the same clone and similar protocol settings on the Ventana BenchMark platform obtained an optimal result. Several parameters could contribute to this difference e.g. sensitivity of the detection systems used and/or impact of other reagents used. It also has to be taken into account that the staining reaction of clone A103 in granulosa cell tumours and other steroid producing cells/structures is related to a cross reaction of the clone to an unknown antigen and e.g. stringent washing conditions in buffer during the staining process might affect this cross reaction. However the crossreaction of clone A103 in steroid producing cells and neoplasias (adrenal cortical and non-epithelial tumours) is widely used in diagnostics and thus the staining performance in these cells is included in the overall assessment of the technical quality of MLA in NordiQC.

The most successful and robust assay for MLA in this assessment was the Ready-To-Use system based on the mAb clone A103 (Dako) giving a pass rate of 91% (39 of 43 laboratories). Of these 74% was assessed as optimal. In comparison, the Ready-To-Use system from Ventana based on the same mAb, gave a pass rate of 56% (23 of 41 laboratories) out of which 17%was assessed as optimal.
Optimal results for the Dako RTU system was typically obtained by the official recommendations given in the package insert using HIER in TRS High pH and EnVision FLEX as detection system. Laboratory modified protocol settings of e.g. adjustment of incubation time of the primary antibody and/or using FLEX+ could also be used to produce optimal results.
For the Ventana RTU system, optimal results could only be obtained by modified and laboratory validated protocol settings using prolonged incubation time of the primary Ab and/or other detection system as UltraView-RED + amplification kit or OptiView +/- amplification instead of the recommendations given in the package inserts. It was observed that HIER CC1 mild (24-32 min.) gave an inferior performance compared to the Ventana recommended standard CC1 (48-64 min.). If mild CC1 was applied, 4 of 17

(24%) protocols provided a sufficient result, whereas by standard or extended CC1, 18 of 22 (82%) protocols were evaluated as sufficient.

Controls

Adrenal gland is a recommendable positive tissue control for MLA when using the mAb clone A103.
A moderate to strong granular cytoplasmic staining reaction must be seen in virtually all 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 staining reaction will thus mimic the specific reaction and eliminate the potential as a reliable positve control. In this assessment, four of the insufficient results showed a false negative staining reaction in the neoplasias, while positive staining reaction was seen in the adrenal cortical epithelial cells due to false positive staining of endogenous biotin by using a biotin-based detection system.
Kidney is recommended as negative tissue control. No staining in the epithelial cells of tubules must be seen. Scattered epithelial cells may show a granular staining reaction caused by lipofuscin.

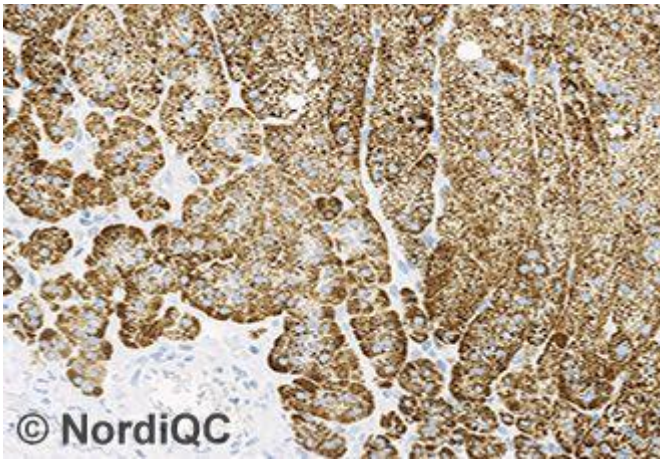


Fig. 1a
Optimal MLA staining of the adrenal gland using the mAb clone A103 diluted 1:100, HIER in TRS High pH 9 for 20 min., a 3-step polymer based detection kit and performed on Autostainer Link, Dako.
Virtually all cortical epithelial cells show a moderate, distinct, granular cytoplasmic staining reaction. No background reaction is seen. Also compare with Figs. 2a – 4a, same protocol.

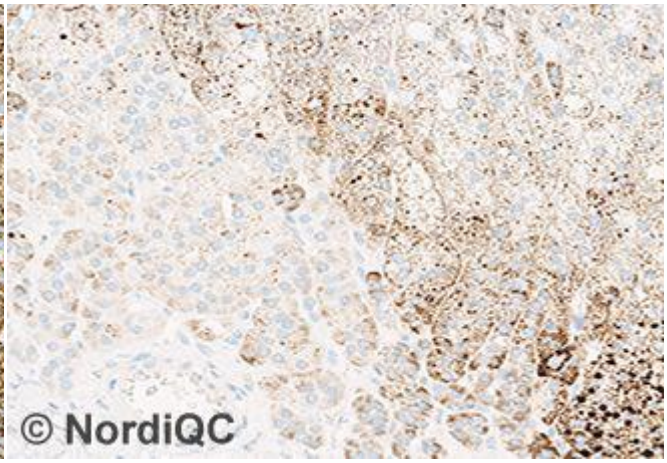


Fig. 1b
MLA staining of the adrenal gland using an insufficient protocol using the mAb clone A103 diluted 1:100, HIER in CC1 pH 8.5 for 32 min., a 3-step multimer based detection kit and performed on BenchMark Ultra, Ventana.
The majority of cortical epithelial cells are demonstrated but the intensity is significantly reduced. Compare with Fig. 1a. – same field.
Also compare with Figs. 2b - 4b – same protocol.

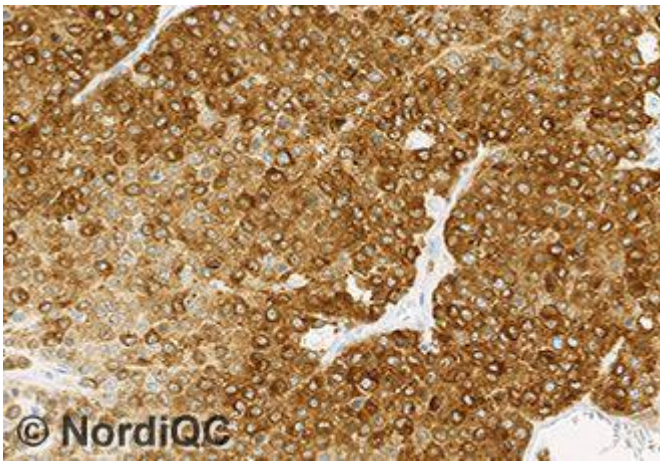


Fig. 2a
Optimal MLA staining of the malignant melanoma tissue core no. 3 using same protocol as in Fig. 1a. All the neoplastic cells show a moderate to strong cytoplasmic staining reaction. No background reaction is seen.

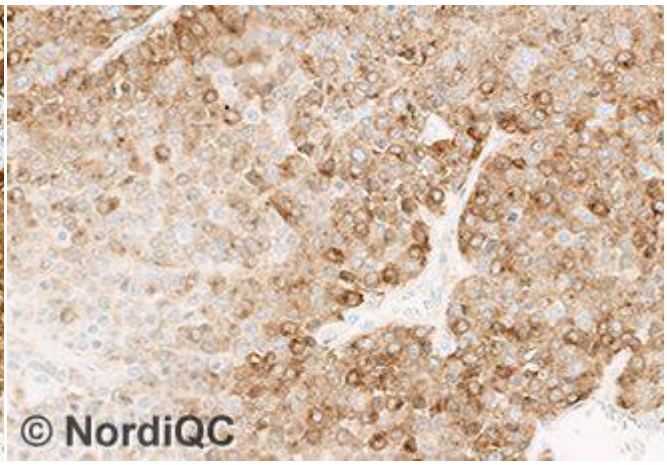


Fig. 2b
MLA staining of the melanoma using same protocol as in Fig. 1b – same field as in Fig. 2b. The majority of neoplastic cells are demonstrated, but the proportion and intensity is reduced compared to the level expected. However, compare with Fig. 3b, same protocol.

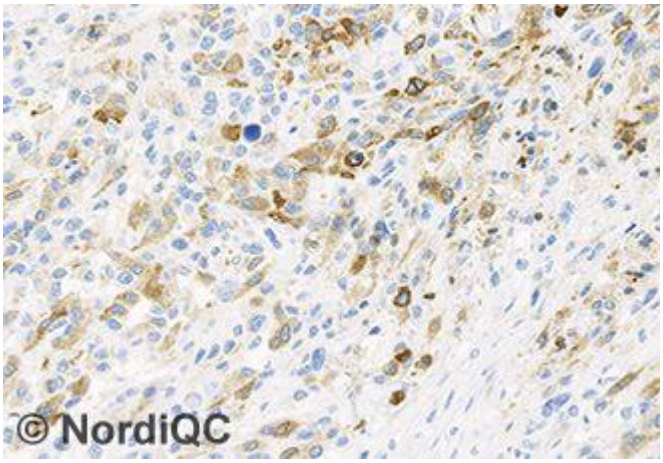


Fig. 3a
Optimal MLA staining of the malignant melanoma tissue core no. 4 using same protocol as in Figs. 1a & 2a. Dispersed neoplastic cells show a weak to moderate cytoplasmic staining reaction. No background reaction is seen.

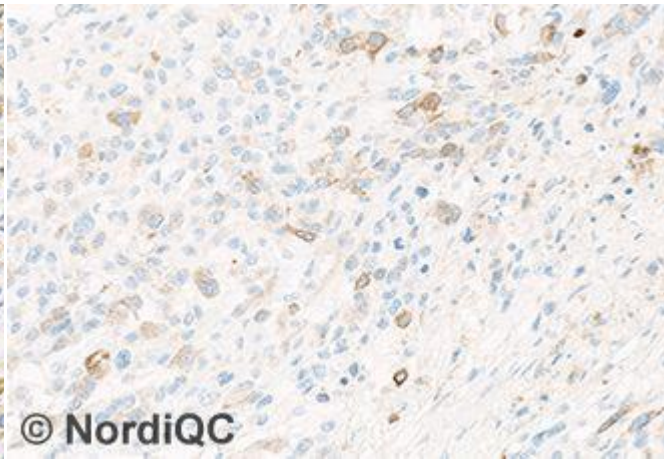


Fig. 3b
Insufficient MLA staining of the malignant melanoma using the same protocol as in Figs. 1b & 2b.The neoplastic cells display only a weak staining reaction. Compare with Fig. 3a – same field.

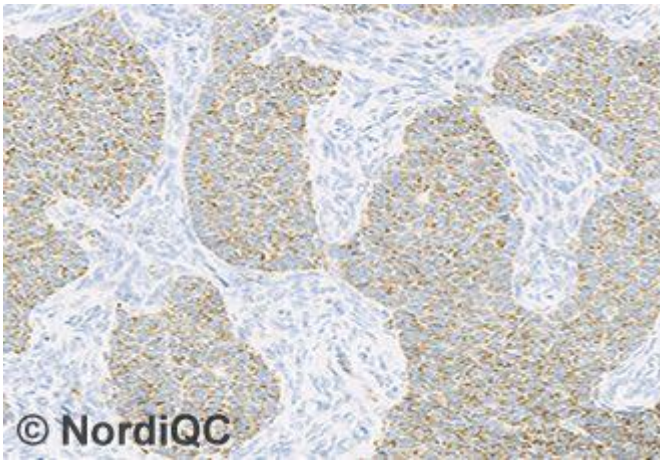


Fig. 4a
Optimal MLA staining of the granulosa cell tumour using same protocol as in Figs. 1a - 3a. The majority of the neoplastic cells show a weak to moderate granular cytoplasmic staining reaction. No background reaction is seen.

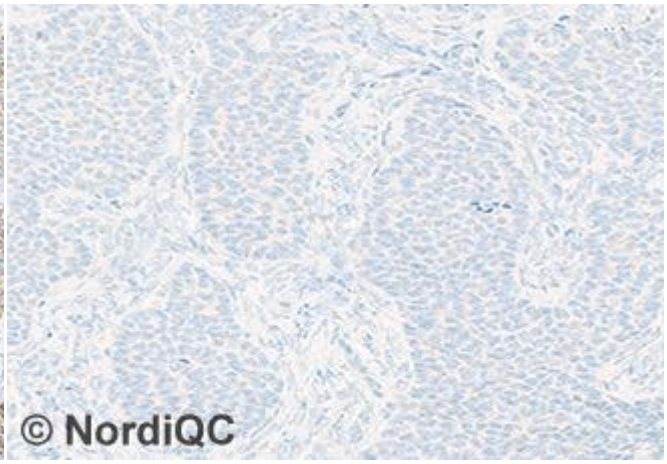


Fig. 4b
Insufficient MLA staining of the granulosa cell tumour using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a. Only scattered neoplastic cells show a weak and equivocal staining reaction.

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