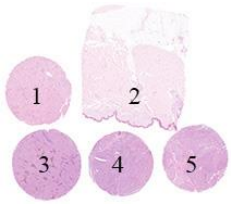
Assessment Run 60 2020

Melan A (MLA)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for MLA, identifying malignant melanomas in the characterization of tumours of unknown origin. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for MLA (see below). This was the first NordiQC assessment of MLA, excluding steroid hormone producing cells and corresponding tumours (only applicable for mAb clone A103), focusing only on intended use in relation to the diagnosis of malignant melanomas.

Material
The slide to be stained for MLA comprised:



1. Kidney, 2. Skin, 3-4. Malignant melanoma, 5. Colon Adenocarcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MLA staining as optimal included:

- A moderate to strong, distinct cytoplasmic staining reaction of virtually all melanocytes in the skin. The dendrites of melanocytes should display a crisp and precise staining reaction.
- A strong, distinct cytoplasmic staining reaction of virtually all neoplastic cells in the malignant melanoma tissue core no 3.
- An at least moderate, distinct cytoplasmic staining reaction of virtually all neoplastic cells in the malignant melanoma tissue core no. 4.
- No staining reaction of epithelial cells in the kidney and of the neoplastic cells in the colon adenocarcinoma.

Participation

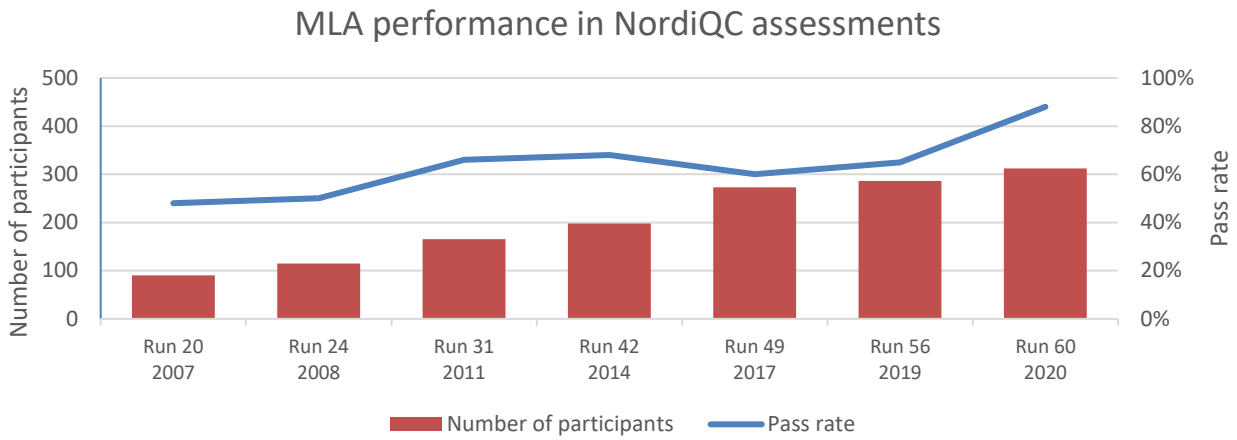
Number of laboratories registered for MLA, run 60	346
Number of laboratories returning slides	316 (91%)

Results
During the assessment a limited number of participants have experienced issues with the circulated NordiQC slides, providing a partial or entire aberrant/false negative staining result in some cases. During the assessment, this observation was taken into account and for MLA, 4 slides were potentially affected. 3 were assessed and one excluded. If performance was characterized by uneven staining or a completely false negative result that could be related to the quality of the slide and not the protocol submitted, this was commented in the individual assessment feed-back.
316 laboratories participated in this assessment. One slide was excluded as mentioned above and three laboratories used an inappropriate antibody. Of the remaining 312 laboratories, 88% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Less successful performance of the mAb clone A103 on the Ventana BenchMark stainer platform
- Too low concentration of the primary antibody or too short incubation time
- Less sensitive detection systems
- Too short efficient Heat Induced Epitope Retrieval (HIER) time

Performance history
This was the 9th NordiQC assessment of MLA. A significant increase in pass rate was observed compared to previous runs (see Table 2). The improved pass rate was most likely impacted by the change of purpose and hereby tissues included in the material circulated.

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



Conclusion

The mAb clones **A103**, **BS52**, **M2-7C10**, and the rmAb clones **EP43, EP1442Y** can all be used to obtain optimal staining results for MLA. The mAb clone **A103** was the most frequently used antibody and although the pass rate was high (88%, 239/273), the proportion of optimal results was low (18%, 49/273). Assays based on the mAb clone A103 require a high level of analytic sensitivity for optimal performance typically using efficient HIER in an alkaline buffer and careful calibration of the antibody titre in combination with a sensitive and specific IHC detection system.
The Ready-to-Use (RTU) system **IR633/IS633** (Dako, Autostainer) and **PA0233/PA0044** (Leica, Bond) based on mAb clone A103, both provided a pass rate of 100% using vendor recommended protocol settings (VRPS) but the proportion of optimal results was low, 7% (1/14) and 29% (2/7), respectively.
The rmAb clone EP43 showed very promising results and was in this assessment the most successful marker for MLA providing the highest proportion of optimal results, in total 97% (22/23). Optimal results were seen on the automated platforms: Ventana Benchmark, Dako Omnis, Leica Bond and Sakura Genie.

Normal skin and melanomas with low MLA expression are recommendable positive tissue controls for MLA. In normal skin, virtually all melanocytes should show a strong cytoplasmic staining reaction and dendrites of melanocytes should be easily visible at low magnification (x10), displaying a crisp and precise staining reaction. 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.

Table 1. **Antibodies and assessment marks for MLA, Run 60**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **A103**	57 19 6 1 1 1 1 1	Dako/Agilent Novocastra/Leica Cell Marque Abcam Biocare Monosan Biogenex Zeta Corporation	21	57	9	0	90%	24%
mAb clone **BS52**	3	Nordic Biosite	3	0	0	0	-	-
mAb clone **M2-7C10**	1	Zytomed	1	0	0	0	-	-
mAb clone cocktail **HMB45+MC-7310 +M2-9E3+T311**	3	Biocare	2	1	0	0	-	-
mAb clone cocktail **HMB45+A103+T311**	1	Diagnostic Biosystems	0	0	0	1	-	-
mAb clone cocktail **M2-7C10+M2-9E3**	1	Thermo F. Scientific	1	0	0	0	-	-
rmAb clone **EP1442Y**	1	Abcam	1	0	0	0	-	-
rmAb clone **EP43**	9 9 1	Nordic Biotite Epitomics Cell Marque	18	1	0	0	100%	95%

Ready-To-Use antibodies								
mAb clone **A103 790-2990**[3]	3	Ventana/Roche	0	0	3	0	-	-
mAb clone **A103 790-2990**[4]	94	Ventana/Roche	6	74	11	3	85%	6%
mAb clone **A103, IR633/IS633**[3]	14	Dako/Agilent	1	13	0	0	100%	7%
mAb clone **A103, IR633/IS633**[4]	56	Dako/Agilent	12	36	7	1	85%	21%
mAb clone **A103, PA0233/PA0044**[3]	7	Leica Biosystems	2	5	0	0	100%	29%
mAb clone **A103, PA0233/PA0044**[4]	10	Leica Biosystems	6	4	0	0	100%	60%
mAb clone **A103, 281M-87/281M-88**	1	Cell Marque	1	0	0	0	-	-
mAb clone **A103, API3114**	1	Biocare	0	0	1	0	-	-
mAb clone **M2-7C10, 281M-97/281M-98**	2	Cell Marque	1	1	0	0	-	-
mAb clone cocktail **HMB45+A103+T311, 904H-08**	1	Cell Marque	0	0	1	0	-	-
mAb clone cocktail **HMB45+A103+T311, 790-4677**	1	Ventana/Roche	0	1	0	0	-	-
mAb clone cocktail **HMB45+MC-7310 +M2-9E3+T311, PM165**	1	Biocare	0	1	0	0	-	-
rmAb clone **BP6086, I1064**	1	Tuling Biotechnology	0	0	1	0		
rmAb clone **EP43, MAD-000695QD-7/N**	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone **EP43, 8319-C010**	3	Sakura Finetek	3	0	0	0	-	-
Total	312		80	194	33	5	-	
Proportion			26%	62%	11%	1%	88%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of MLA, Run 60
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **A103**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1, Ventana) (5/40)*, Target Retrieval Solution (TRS, Dako) pH 9 (3-in-1) (8/21) or Bond Epitope Retrieval Solution 2 (BERS2, Leica) (8/20), as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 61 of 67 (91%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **BS52**: Protocols with optimal results were based on HIER in TRS High pH (3-in-1) (Dako) (1/1), CC1 (Ventana) (1/1) or Tris-EDTA/EGTA pH 9 (1/1) as retrieval buffer. The mAb was diluted 1:100 in all protocols applied.

mAb clone **M2-7C10**: One protocol with an optimal result was based on HIER in a pressure cocker using Citrate pH 6 as retrieval buffer. The mAb was diluted 1:75 and Zytomed (POLHRP-100) was used as the detection system.

mAb clone cocktail **HMB45+MC-7C10+M2-9E3+T311**: Protocols with optimal results were based on HIER using Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/1) or CC1 (Ventana) (1/2) as retrieval buffer. The mAb was diluted in the range of 1:75-1:500.

mAb clone cocktail **MC-7C10+M2-9E3**: One protocol with an optimal result was based on HIER using BERS1 (Bond, Leica) (1/1) as retrieval buffer. The mAb was diluted 1:200 and Bond Refine (Leica) was used as the detection system.

rmAb clone **EP1442Y**: One protocol with an optimal result was based on HIER using TRS High pH (3-in-1) (Dako). The mAb was diluted 1:200 and Envision Flex (Dako) was used as the detection system.

rmAb clone **EP43**: Protocols with optimal results were based on HIER using TRS High pH (3-in-1) (Dako) (5/5), CC1 (Ventana) (5/6), CC1 (Ventana) followed by Protease 3 (Ventana) (6/6), BERS2 (Leica) (1/1) or Tris-EDTA/EGTA pH9 (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:25–1:100. Using these protocol settings, 19 of 19 (100%) laboratories produced a sufficient staining result.

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

Concentrated antibodies	**Dako Autostainer**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**			**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **A103**	0/5** (0%)	0/1	6/9 (66%)	-	5/34 (15%)	-	-	7/17 (41%)	0/1
rmAb clone **EP43**	-	-	5/5 (100%)	-	5/6 (83%)	6/6 (100%)	-	1/1	-

* 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**, product no. **790-2990**, Ventana/Roche, BenchMark GX/XT/ULTRA:
Protocols with optimal results were typically based HIER using CC1 (efficient heating time 64 min. at 95-100°C), 24-32 min. incubation of the primary Ab and OptiView (760-700) with or without amplification (760-099 / 860-099) as detection system. Using these protocol settings, 13 of 14 (93%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **A103**, product no. **IR633/IS633,** Dako/Agilent, Autostainer Classic/Link:
Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1), efficient heating time for 10-20 min. at 97-99°C, 20-30 min. incubation of the primary Ab and EnVision Flex as detection system. Using these protocol settings, 20 of 20 (100%) laboratories produced a sufficient staining result.

mAb clone **A103,** product.no. **PA0233**, Leica Biosystems, BOND III/MAX:
Protocols with optimal results were based on HIER in BERS2 (efficient heating time 20-30 min. at 99-100°C), 15-30 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 16 of 16 (100%) laboratories produced a sufficient staining result.

rmAb clone **EP43**, product no. **8319-C010**, Sakura Finetek, Tissue-Tek Genie:
Protocols with optimal results were based on HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer (efficient heating time 45-60 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for MLA for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT/GX mAb A103 **790-2990**	0/3	0/3	85% (80/94)	6% (6/94)
Dako AS mAb A103 **IR633/IS633**	100% (14/14)	7% (1/14)	92% (11/12)	17% (2/12)
Leica Bond III/MAX mAb A103 **PA0233/PA0044**	100% (7/7)	29% (2/7)	100% (10/10)	60% (6/10)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In previous NordiQC MLA assessments, laboratories using the mAb clone A103 have been assessed on their ability to detect both the specific MLA and the unknown cross-reacting protein in steroid hormone producing cells and corresponding tumours, whereas laboratories using other clones have been assessed on their ability to detect MLA only. Although the MLA assessment (Run 56) was planned to be executed as a combined "**melanoma/melanocyte – steroid hormone**" assessment, it was decided by NordiQC primarily to focus the assessment and report on the specific MLA data, excluding the steroid hormone producing normal tissue (e.g. adrenal gland) and tumor (e.g. ovarian granulosa cell tumor) and only address this focus in forthcoming runs. Therefore, this assessment (Run 60) only intended to detect MLA in malignant melanomas, identifying this entity in the diagnostic workup of tumors of unknown origin.

In concordance with the previous NordiQC assessments for MLA, the prevalent feature of an insufficient staining was a general too weak or false negative staining reaction of cells and structures expected to be demonstrated. This was observed in 87% (33/38) of the insufficient results and was only seen in protocols based on the mAb clone A103 either as single antibody or as part of a cocktail. In the remaining protocols, 13% (5/38) of the insufficient results were caused by poor signal to noise or false positive reactions mainly related to the use of MACH4 (Biocare) or PolyVue Plus (Diagnostic Biosystems) as the detection system. In general, almost all laboratories could detect MLA in the malignant melanoma, tissue core no. 3, whereas demonstration of MLA in the malignant melanoma, tissue core no. 4, and in the melanocytes of the normal skin (both the proportion and intensity) was more challenging requiring an optimally calibrated protocol.

The mAb clone A103 was the most widely used antibody for demonstration of MLA and applied by 88% (273 of 312) of the laboratories (see Table 1). Used as concentrated format within laboratory developed (LD) assays, 90% (78/87) of the protocols were assessed as sufficient (optimal or good). Optimal results could be obtained on the automated IHC platforms from the three major vendors (see Table 3). Although data is limited, and for protocols applied on the platforms outlined in Table 3, the most prevalent feature of an insufficient result was use of a low sensitive detection system (e.g. Envision Flex/Dako or UltraView/Ventana) or use of a 3-step polymer detection system (Bond Refine Red, DS9390, Leica) in combination with protocol settings giving a low analytical sensitivity as too short HIER time, too short incubation time or too diluted primary antibody. Applying these protocol settings, all protocols (7/7) were assessed as borderline. In comparison, all assays providing an optimal result were based on protocol settings giving a high level of analytical sensitivity e.g. by using efficient HIER in an alkaline buffer, careful calibration of the primary antibody to the selected platform and a sensitive 3-step polymer/multimer detection system with or without amplification.
As observed in the previous Run 56, the proportion of optimal results was significantly influenced by the automated IHC platforms applied (see Table 3). On the Dako Omnis platform, 66% (6/9) of the protocols were assessed as optimal, whereas the proportion of optimal staining results on the Leica Bond and Ventana BenchMark platforms was 41% (7/17) and 15% (5/34), respectively. These data indicate, that the mAb clone A103 is challenging and especially for participants using a Ventana BenchMark platform. For this particular platform, optimal results could be obtained using HIER in CC1 (64min.), dilution of the antibody in the range of 1:20-1:50, incubation in primary antibody for 32 min. and use of OptiView with or without amplification or UltraView with amplification as the detection systems. However, there is a narrow threshold between optimal and suboptimal results as a relatively large group of eleven laboratories were obtaining a sufficient mark (good) applying similar protocol settings.
Using the "optimal" dilution range as described above for mAb clone A103, none (5/5) of the protocols applied on the semi-automated Dako Autostainer provided an optimal result and was most likely caused by use of the low sensitive 2-step detection system EnVision Flex (Dako). Beyond the results in Table 3, two protocols were assessed as optimal applying efficient HIER in TRS pH 9 (20 min.), mAb clone A103 diluted

1:200, incubation in primary antibody for 20-30 min. and the use of a sensitive 3-step detection system Envision Flex+ (Dako), indicating that all parameters must be taken into account and especially the antibody titre carefully adjusted for optimal performance. Applying similar protocol settings, a higher concentration of the mAb A103 is needed to produce an optimal result on the Omnis platform compared to the Autostainer, typically using the primary antibody in the dilution range of 1:25-1:50 and 1:100-1:200, respectively.
On the Bond platforms (Leica), the proportion of optimal staining results with mAb clone A103 was 41% (7/17) (see Table 3). Laboratories typically used HIER in BERS2 for 20-30 min., diluted the antibody in the range 1:50-1:100, incubated in primary antibody for 30 min. and used Bond Refine/DAB(DS9800) or Bond Refine/Red (DS9390) as detection systems.

The rmAb clone EP43 has shown consistent and promising results in previous Runs (40 and 56) but also in the present run 60. Used within a LD assay, 100% (19/19) of the laboratories obtained sufficient staining results of which 95% (18/19) were assessed as optimal. As shown in Table 3, optimal staining results could be obtained on the Leica Bond, Ventana BenchMark and Dako Omnis platforms, whereas no laboratories used the rmAb clone EP43 on the Dako Autostainer. The rmAb clone EP43 is very robust and seems to be the most sensitive marker for demonstration of MLA in malignant melanomas. Several parameters could be used to obtain an optimal result including HIER in alkaline buffer (e.g. CC1/Ventana), combined retrieval (HIER in CC1 followed by protease 3/Ventana), use of both 3-step detection systems (e.g. OptiView/Ventana) or 2-step detection systems (e.g. UltraView/Ventana) as long as the primary antibody was diluted in the range of 1:25-100. This observation emphasizes the robustness of the antibody and might be helpful for participants striving to obtain a correct and high level of analytic sensitivity using the more challenging mAb clone A103 in the diagnostic workup of malignant melanomas. However, attention must be put to the purpose of the test and if the assay should be used to characterize steroid producing neoplasm, the rmAb clone EP43 will not react with these tumors.

In total, 63% (196/312) of the laboratories used a RTU format. The most widely used RTU systems for MLA were the Ventana 790-2990, Leica PA0233/PA0044 and Dako IR633/IS633 systems, all based on the mAb clone A103. Following VRPS, the Dako IR633/IS633 system tailored for the Dako Autostainer had a high pass rate of 100% (14/14) but only 7% (1/14) were giving an optimal mark (see Table 4). Using Laboratory Modified Protocol Settings (LMPS) the pass rate decreased to 92% (11/12) with 17% (2/12) being optimal. The main reason for the low proportion of optimal results, is most likely related to the recommended detection system (Envision Flex) used in virtually all protocols (25/26). Based on the results obtained from the LD assays, the mAb clone A103 require highly sensitive protocol settings including the use of a 3-step polymer detection system as Envision Flex+ for optimal performance.
A significant proportion of participants used the RTU format IR633/IS633 on the Dako Omnis most likely due to lack of a RTU alternative to this instrument. In total, 37 laboratories applied this RTU format on the Dako Omnis, providing an overall pass rate of 84% (31/37) of which 27% (10/37) were optimal. Using similar protocol settings as recommended by Dako for the Dako Autostainer, the proportion of sufficient results decreased to 50% (6/12) with only one being optimal. As reported in the previous assessment (Run 56), the use of modified and optimized protocol settings, especially by use of a 3-step polymer detection system (Envision Flex+), resulted in an improved pass rate of 100% (19/19) and 42% (8/19) were assessed as optimal. These data, and in concordance with previous findings, show that "direct" transfer of original Autostainer protocols to the Dako Omnis should be avoided and requires a careful validation by the laboratory. Adjustments of key parameters as HIER, incubation time of the primary antibody and choice of detection system is typically needed to provide the correct analytical accuracy for the IHC test.

97 laboratories used the RTU format (790-2990) of mAb clone A103 from Ventana. Only 3 participants used the recommended protocol settings of which none were assessed as sufficient (see Table 4). Overall, the performance of the RTU system was challenged by suboptimal results as only 6% (6/97) protocols gave an optimal result. Also, 18% (17/97) of the protocols were assessed as insufficient characterized by too weak or false negative results and typically caused by application of low sensitive protocol settings - often used in combination as reduced HIER time in CC1, reduced incubation time in primary antibody and/or use of UltraView-AP/HRP as the detection system. This was observed in 82% (14/17) of the protocols. The recommended protocol settings are based on the use of UltraView-AP (without amplification) as detection system, but among 18 laboratories using this detection system with various protocol modifications, the pass rate was only 44% (8/18) and none were optimal. For laboratories using UltraView-AP with amplification, a pass rate of 100% (16/16) was achieved but none were assessed as optimal. This result is somewhat surprising, as protocols based on this detection system has previously shown to be successful and highly sensitive, providing a high proportion of optimal results (see assessment Run 49 2017). However as seen in Run 56, the protocols provided the expected and satisfactory level of analytical sensitivity and staining reaction, but slides had a concurrent excess

background staining, challenging the interpretation which resulted in a downgrade from an optimal mark to good. For detailed analysis and discussion of the problems – please see assessment Run 56 (2019). For participants using OptiView with or without amplification as the detection system, the pass rate was 100% (28/28) of which 18% (5/28) were giving an optimal mark (for optimal protocol settings see above). These data are in concordance with the findings observed for LD assays, supporting that the mAb clone A103 is challenging and require extensive optimization for Benchmark platforms.

In this assessment, all protocols (17/17) based on the Leica RTU format PA0233/PA0044 of mAb clone A103 provided a sufficient result (see Table 4). Applying both VRPS and LMPS, the proportion of optimal results was significantly higher using the RTU format PA0233/PA0044 (Leica) compared to the performance of the RTU formats from the Dako (IR633/IS633) and Ventana (790-2990) - 47% (8/17), 12% (3/26) and 6% (6/97), respectively. The results obtained with the RTU format PA0233/PA0044 on the Leica Bond is similar to results obtained within a LD-assay applying the same platform, and as seen in this particular run, the mAb clone A103 seems less challenging on Bond instruments.

This was the ninth NordiQC assessment of MLA. A significant increase in the pass rate was observed (see Table 2) from 65% in Run 56 (2019) to 88% in Run 60 (2020) and most likely due to less challenging material circulated to the participants and change of main purpose of MLA IHC only focusing on melanomas and not steroid producing cells/tumours. The most important parameters influencing the final result in negative direction were the use of low sensitive protocol settings e.g. too short HIER and/or use of a 2-step polymer/multimer detection system.

Controls

Normal skin and melanomas with low MLA expression are recommendable positive tissue control for MLA. In normal skin, virtually all melanocytes should show strong positive reaction in the cytoplasm. The dendrites of melanocytes should display a crisp and precise staining reaction. Kidney is recommended as negative tissue control and no staining should be seen in the epithelial cells of the tubules. Scattered epithelial cells may show a granular staining reaction caused by lipofuscin.

The purpose of this assessment was evaluation of the technical performance and level of analytical sensitivity in the context of identification of melanomas in the diagnostic workup of tumors of unknown origin. However, as the vast majority of laboratories applied the mAb clone A103 either within a LD-assay or as a RTU format, and the proportion of optimal results were very low (18%, 49/273), it is advisable to include adrenal gland to the in-house on slide control to ensure that the correct level of analytic sensitivity is attained for the chosen assay. In adrenal gland, the mAb A103 "cross-react" with steroid producing cells and virtual all cortical cells should display a moderate to strong, distinct granular staining reaction.

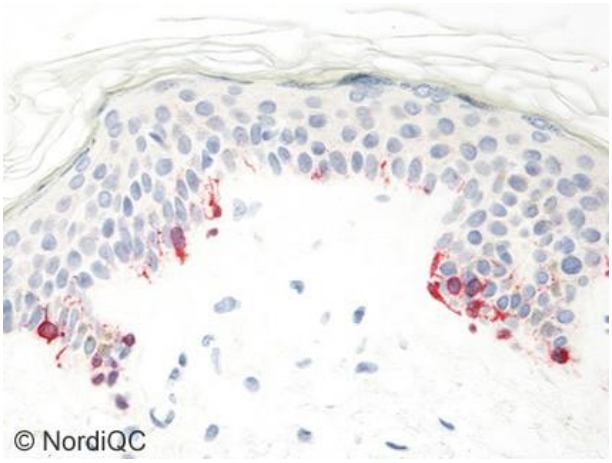


Fig. 1a (x400)
Optimal staining for MLA of the skin using the mAb clone A103 as a concentrate (1:50), efficient HIER in an alkaline buffer (BERS2, Leica) and a 3-step polymer based detection system (Bond Refine Red, DS9390, Leica) - same protocol used in Figs. 2a - 5a. Virtually all melanocytes show a strong cytoplasmic staining reaction and melanocytic dendrites are weak to moderate labelled.

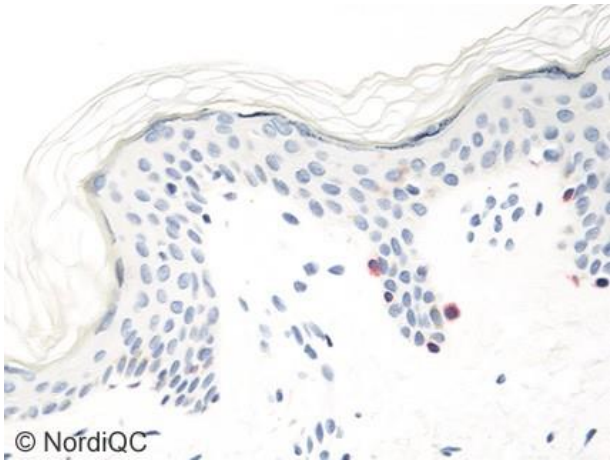


Fig. 1b (x400)
Insufficient staining for MLA of the skin using the mAb A103 as concentrate (1:60), too short HIER time (10 min.) in BERS2 in combination with too short incubation time in primary Ab (15 min.) and Bond Refine Red as the detection system - same protocol used in Figs. 2b – 5b. Only dispersed melanocytes are demonstrated, the intensity is significantly reduced, and melanocytic dendrites are negative. Compare with Fig. 1a.- same field.

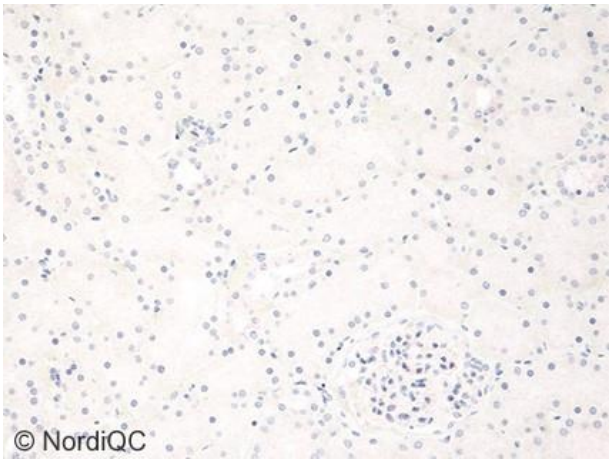


Fig. 2a (x200)
Optimal MLA staining of the kidney using same protocol as in Fig. 1a. The epithelial cells of tubules display the expected negative staining reaction.

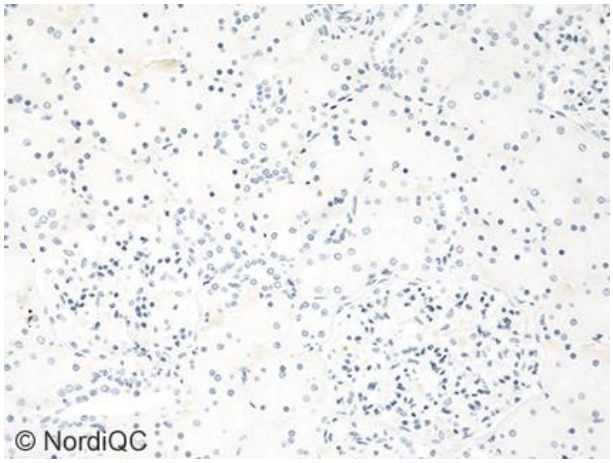


Fig. 2b (x200)
MLA staining of the kidney using same protocol as in Fig. 1b – same field as in Fig. 2a. Although the tubules show the expected staining pattern, the protocol applied is generally unreliable due to protocol settings giving a low analytical sensitivity, risking misdiagnosis of melanomas - compare Figs. 3a-4b.

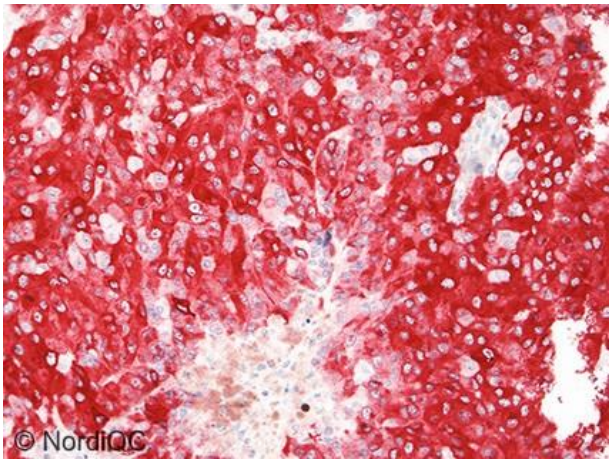


Fig. 3a (x200)
Optimal MLA staining of the malignant melanoma, tissue core no. 3 (high-level expressor), using same protocol as in Figs. 1a and 2a. All neoplastic cells show a strong cytoplasmic staining reaction.

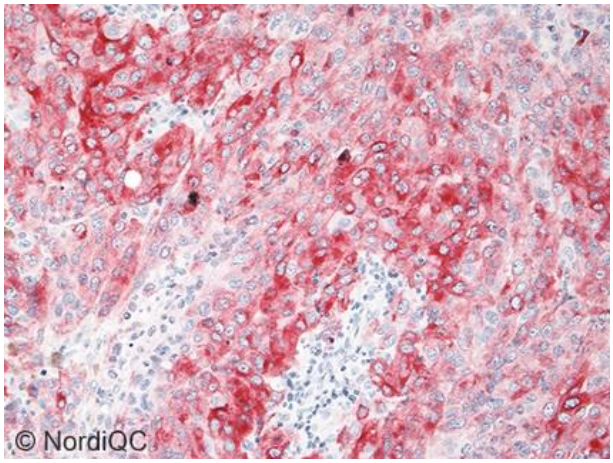


Fig. 3b (x200)
Insufficient MLA staining of the malignant melanoma, tissue core no. 3 (high-level expressor), using the same protocol as in Figs. 1b and 2b. The majority of the neoplastic cells show a cytoplasmic staining reaction, but the intensity is significantly reduced. Compare with Fig. 3a – same field.

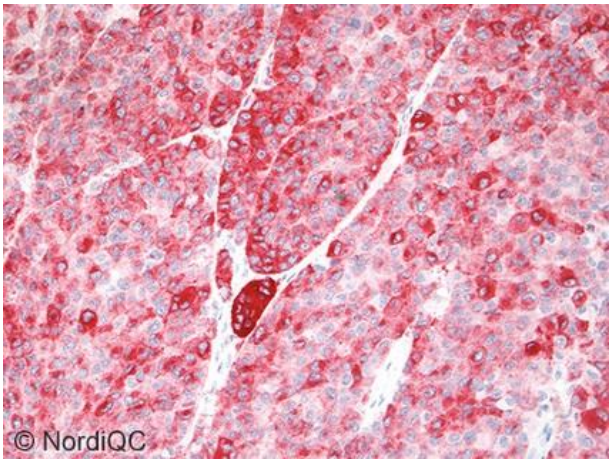


Fig. 4a (x200)
Optimal MLA staining of the malignant melanoma, tissue core no. 4, using same protocol as in Figs. 1a-3a. Virtually all neoplastic cells display a moderate to strong cytoplasmic staining reaction.

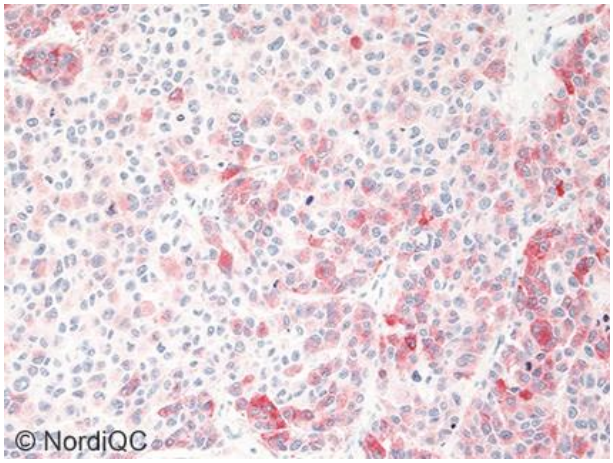


Fig. 4b (x200)
Insufficient MLA staining of the malignant melanoma, tissue core no. 4, using the same protocol as in Figs. 1b - 2b. The majority of the neoplastic cells are false negative or only display a weak to moderate cytoplasmic staining reaction. Compare with Fig. 4a – same field.

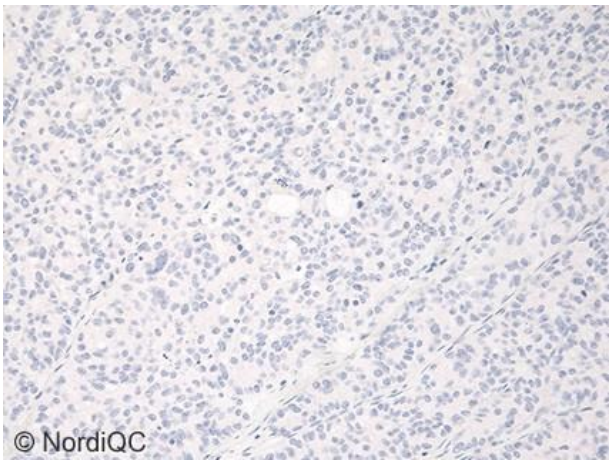


Fig. 5a (x200)
Optimal MLA staining of the colon adenocarcinoma using same protocol as in Fig. 1a-4a. The neoplastic cells display the expected negative staining reaction.

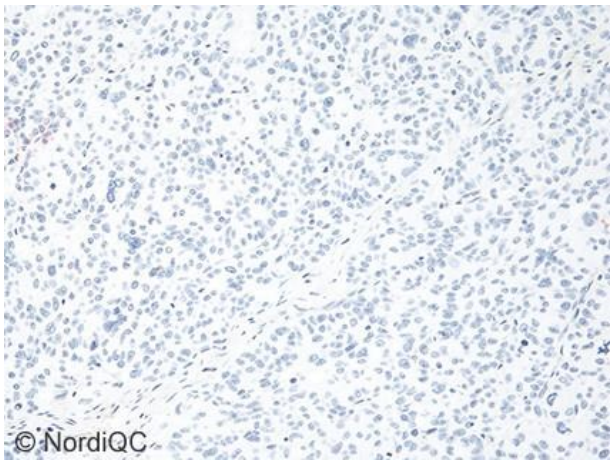


Fig. 5b (x200)
MLA staining of the colon adenocarcinoma using same protocol as in Fig. 1b-4b. Although the neoplastic cells are negative, and as mentioned in Fig. 2b, the protocol is unreliable due to protocol settings giving a low analytical sensitivity.

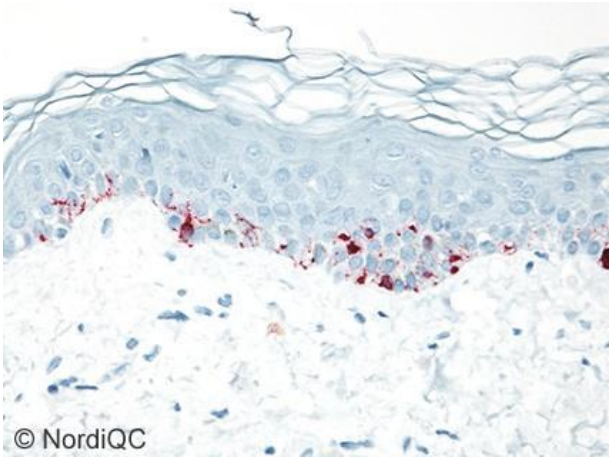


Fig. 6a (x400)
Optimal staining for MLA of the skin using the rmAb EP43 as a concentrate (1:25), combined retrieval with HIER in CC1 /Protease 3 (Ventana) and a 2-step multimer based detection system (UltraView, 760-501, Ventana) - same protocol used in Fig. 6b. Virtually all melanocytes display the expected staining reaction.

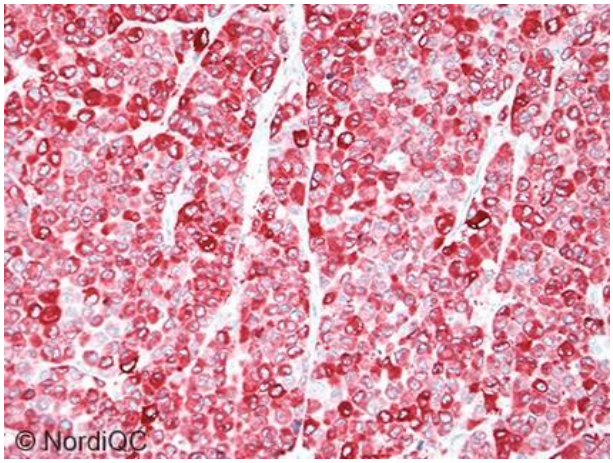


Fig. 6b (x200)
Optimal MLA staining of the malignant melanoma, tissue core no. 4, using same protocol as in Fig. 6a. All neoplastic cells show a moderate to strong staining reaction. The rmAb EP43 is very robust for demonstration of MLA and several parameters could be used for optimal performance including use of the less sensitive detection system UltraView. This pattern was impossible to obtain with the mAb A103 using similar protocol settings.

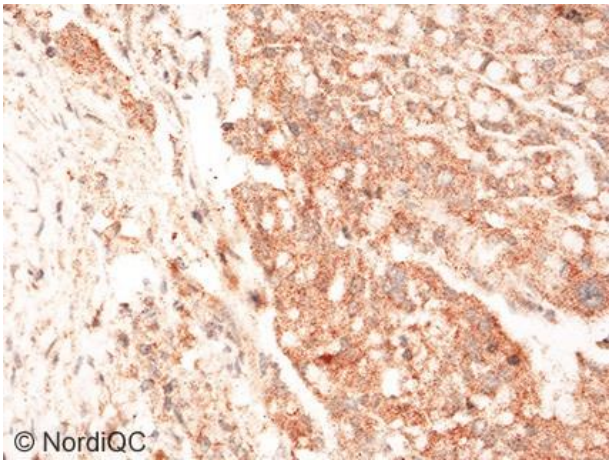


Fig. 7a (x200)
Insufficient staining of the colon adenocarcinoma. The protocol was based on the mAb cocktail HMB45+A103+T311 used within a LD-assay, HIER in Montage Citrate Antigen Retrieval Solution (Diagnostic Biosystems) and PolyVue Plus (Diagnostic Biosystems) as the detection system – same protocol used in Fig. 7b. The neoplastic and stromal cells are false positive, displaying a granular staining reaction. Compare with optimal staining in Fig. 5a.

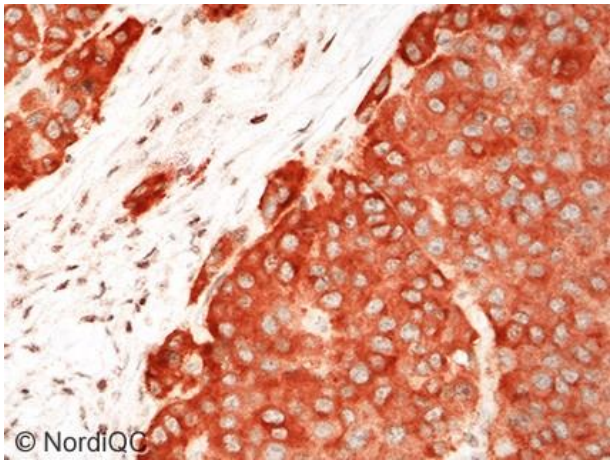


Fig. 7b (x400)
Insufficient MLA staining of the malignant melanoma, tissue core no. 4, using the same protocol as in Fig.7a. Although the neoplastic cells are demonstrated, the interpretation is difficult due to a false positive staining of stromal cells.

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