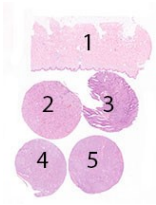
Assessment Run 74 2025
Melan A (MLA)

Updated 11.07.2025

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

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 5.
- 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.

KEY POINTS FOR MLA IMMUNOASSAYS
- A historically low pass rate of 34% was observed during Run 74 with only 9.5% being optimal.
- The mAb clone **A103** was used by 90% of all participants either as a concentrate or RTU product.
- The mAb clone **A103** seemed to have less affinity for low-level MLA expression in malignant melanomas compared to other clones.
- The LD assays using mAb clone **BS52** and rmAb clone **EP43** had the highest pass rate and optimal result could be obtained on all main fully automated IHC platforms.

Participation

Number of laboratories registered for MLA, run 74	430
Number of laboratories returning slides	391 (91%)

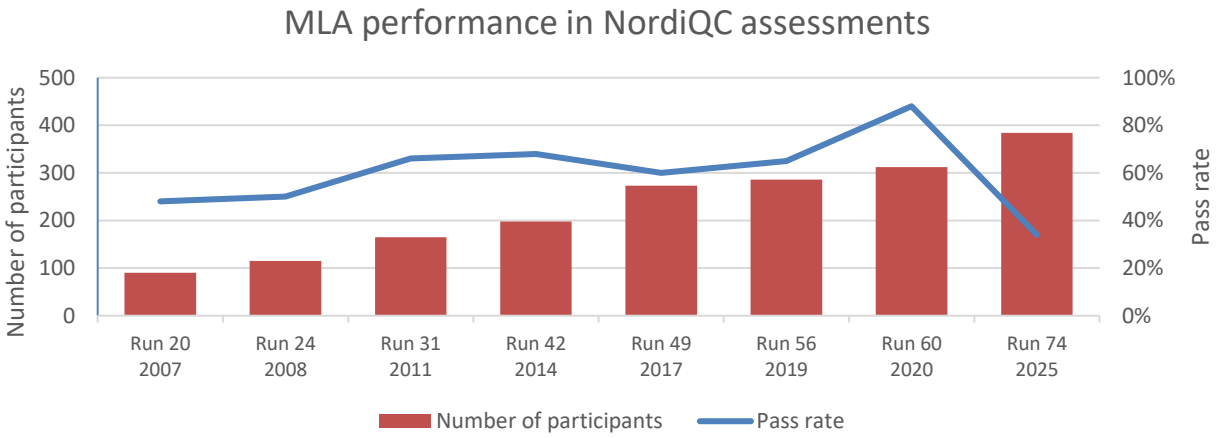
Results
At the date of assessment, 91% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

391 laboratories participated in this assessment, however 7 laboratories submitted inappropriate antibodies, and the data were not included in this report. 34% achieved a sufficient mark (optimal or good). Table 1a-c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining were:
- Less successful performance of the mAb clone A103
- 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 10th NordiQC assessment of MLA. A significant decrease in pass rate was observed compared to previous runs (see Graph 1). The decline pass rate was most likely impacted by the use of mAb clone A103 challenged by the low level of MLA in one of the neoplastic tissues in the material circulated.

Graph 1. **Proportion of sufficient results for MLA in the ten NordiQC runs performed**



Control
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 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 reaction should be seen in the epithelial cells of the tubules. Scattered epithelial cells may show a granular staining reaction caused by lipofuscin.
It is advisable to include adrenal gland to the in-house on slide control when using the mAb clone A103 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.

Conclusion
The mAb clone **BS52** and the rmAb clones **EP43, EP1442Y** and **QR138** can all be used to obtain optimal staining results for MLA. The mAb clone **A103** was the most frequently used antibody clone and was used by 90% of the laboratories, however the levels of sufficient protocols were very low (26%) and only very few produced an optimal result (1.5%). 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 submitted protocols with the A103 clone which in previous run has shown difficulties with detecting low-levels of MLA expression, this feature was revealed extensively in this assessment run 74 and significantly affected the general pass. In contrast, emerging clones like the rmAb EP43 and mAb BS52 have demonstrated superior robustness on automated IHC platfoms and still with diagnostic reliability, providing laboratories with promising alternatives. This assessment underscores the need for careful selection of antibody clone, meticulous calibration, use of HIER in an alkaline buffer and 3-layer detection systems to ensure accurate and reliable identification of melanomas. Laboratories and vendors of IHC systems should prioritize to optimize, validate and apply the most robust and effective IHC assays to improve diagnostic precision and reduce the risk of misdiagnosis.
Internal studies performed at NordiQC including melanomas and non-melanomas revealed a quite high and significant difference in the diagnostic sensitivity for melanomas and associated H-scores comparing LD IHC assays based on the e.g. rmAb clone EP43 to a widely used RTU IHC assay applied within VRPS. Using an H-score of ≥50 as positive cut-off level, 94% of melanomas were positive with rmAb clone EP43 (median H-score of 260) compared to 82% for mAb clone A103 (median H-score 160) – see page 8.

Table 1a. **Overall results for MLA, run 74**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	93	28	25	40	-	57%	30%
Ready-To-Use antibodies	291	10	67	212	2	27%	4%
Total	384	38	92	252	2		
Proportion		9.5%	24%	66%	0.5%	34%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for MLA, run 74**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **A103**	41 11 5 2 1 1	Dako/Agilent Leica Biosystems Cell Marque Bio SB Quartett Zeta Corporation	3	20	38	-	38%	5%
mAb clone **BS52**	5	Nordic Biosite	5	-	-	-	100%	100%
mAb clone **M2-7C10**	1 1	Cell Marque Bio SB	-	1	1	-	-	-
mAb clone cocktail **HMB45+M2-7C10+M2-9E3+T3**	1	Biocare Medical	1	-	-	-	-	-
mAb clone **IHC418**	1	GenomeMe	-	1	-	-	-	-
rmAb clone **EP43**	11 8 1	Nordic Biosite Epitomics Cell Marque	17	3	-	-	100%	85%
rmAb clone **EP1422Y**	1	Abcam	1	-	-	-	-	-
rmAb clone **QR138**	1	Quartett	1	-	-	-	-	-
clone **HGR-530**	1	Bio Highgrade	-	-	1	-	-	-
Total	93		28	25	40	-		
Proportion			29%	27%	44%	-	56%	

1) Proportion of sufficient stains (optimal or good). (≥5 assessed protocols).
2) Proportion of Optimal Results.

Table 1c. **Ready-To-Use antibodies and assessment marks for MLA, run 74**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **A103 790-2990 (VRPS)[3]**	13	Ventana/Roche OptiView	-	3	10	-	23%	0%
mAb clone **A103 790-2990 (VRPS)[3]**	15	Ventana/Roche UltraView Red	-	5	10	-	33%	0%
mAb clone **A103 790-2990 (LMPS)[4]**	110	Ventana/Roche	1	8	100	1	8%	1%
mAb clone **HMB45+A103+T311 790-4677**	2	Ventana/Roche	-	2	-	-	-	-
mAb clone **A103 IR/IS633 (VRPS)[3]**	15	Dako/Agilent	-	5	10	-	33%	0%
mAb clone **A103 IR/IS633 (LPMS)[4]**	80	Dako/Agilent	1	40	39	-	51%	1%
mAb clone **A103 PA0233/PA0044 (VRPS)[3]**	26	Leica Biosystems	-	1	25	-	4%	0%
mAb clone **A103 PA0233/PA0044 (LMPS)[4]**	13	Leica Biosystems	-	1	12	-	8%	0%
mAb clone **A103 281M-87/281M-88**	1	Cell Marque	-	-	1	-	-	-
mAb clone **A103 API3114**	1	Biocare Medical	-	1	-	-	-	-
mAb clone **A103 AM361-5M/10M**	1	BioGenex	-	1	-	-	-	-
mAb clone **BY237 BFM-0191**	1	Bioin Biotechnology	-	-	1	-	-	-
mAb clone **C5G8 CMM-0131**	1	Celnovte	1	-	-	-	-	-
mAb clone **A103 GM7196**	1	Gene Tech	-	-	1	-	-	-
mAb clone **A103 P-M002-70**	1	Quartett	-	-	1	-	-	-
mAb clone **A103 PDM153-10M**	1	Diagnostic Biosystems	-	-	-	1	-	-
mAb clone **MX118 MAB-1033**	1	Fuzhou Maixin	1	-	-	-	-	-
mAb clone **M2-7C10 MSG056**	1	Zytomed Systems	-	-	1	-	-	-
rmAb clone **EP43 8319-C010**	3	Sakura Finetek	3	-	-	-	-	-
rmAb clone **EP43 MAD-000695QD**	2	Master Diagnostica	2	-	-	-	-	-
rmAb clone **BP6086 I10642E**	1	BioLynx Biosystems	1	-	-	-	-	-
Clone **134H9E9 PA231**	1	Abcarta	-	-	1	-	-	-
Total	291		10	67	212	2		
Proportion			3%	23%	73%	1%	26%	

1) Proportion of sufficient results (optimal or good). (≥5 assessed 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 assessed 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 assessed protocols)

Detailed analysis of MLA, Run 74
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/Roche) (1/27)* or Target Retrieval Solution (TRS, Dako/Agilent) pH 9 (3-in-1) (2/5) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 and using either FLEX+ (Dako/Agilent) or OptiView with amplification (Ventana/Roche) as detection system. Using these protocol settings, 12 of 16 (75%) 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/Agilent) (1/1), CC1 (Ventana/Roche) (3/3) or Bond Epitope Retrieval Solution (BERS2, Leica Biosystems) (1/1) as retrieval buffer. The mAb was diluted 1:100-400 with optimal result in all 5 protocols applied.

rmAb clone **EP43**: Protocols with optimal results were based on HIER using TRS High pH (3-in-1) (Dako/Agilent) (5/5), CC1 (Ventana/Roche) (5/7), CC1 (Ventana/Roche) followed by Protease 3 (Ventana/Roche) (6/6) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (1/2) as retrieval buffer. The rmAb was diluted in the range of 1:25–1:100. Using these protocol settings, 20 of 20 (100%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for MLA for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**			**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + P3	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **A103**	2/5** (40%)	-	0/11	-	1/27 (4%)	-	-	0/15	0/1
mAb clone **BS52**	-	-	1/1	-	3/3	-	-	1/1	-
rmAb clone **EP43**	-	-	5/5 (100%)	-	5/7 (71%)	6/6 (100%)	-	1/2	-

* 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)
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra Plus
3) Bond III, Prime, Max

Ready-To-Use antibodies and corresponding systems
mAb clone **A103**, product no. **790-2990**, Ventana/Roche, BenchMark GX/XT/ULTRA/Ultra plus:
Only one protocol received an optimal result and was based on HIER using CC1 (efficient heating time 64 min. at 99°C), 32 min. incubation of the primary Ab and OptiView (760-700) with amplification (760-099 / 860-099) as detection system. Using these protocol settings, 2 of 4 (50%) laboratories produced a sufficient staining result (optimal or good).
The product was used by 1 laboratory on a non-intended platform. These data are not included here.

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 3 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 (in Table 1 LMPS also includes off label use on deviant IHC stainers).

Table 3. **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
Dako Autostainer[1] mAb clone A103 **IR/IS633**	33% (5/15)	(0/15)	80% (4/5)	(0/5)
Ventana BenchMark[2] OptiView/UltraView DAB mAb clone A103 **790-2990**	23% (3/13)	(0/13)	8% (6/80)	1% (1/80)
Ventana BenchMark[2] UltraView Alkaline Phosphatase Red mAb clone A103 **790-2990**	33% (5/15)	(0/15)	7% (2/29)	(0/29)
Leica Bond[3] mAb clone A103 **PA0233/PA0044**	4% (1/26)	(0/26)	8% (1/12)	(0/12)

* 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 are integrated.
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra Plus
3) Bond III, Prime, Max

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, in the previous run 60 and this assessment run 74, the results were evaluated with primary intended purpose 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 structures expected to be demonstrated. This was observed in 78% (298/384) of the insufficient results and was mainly seen in protocols based on the mAb clone A103. In the remaining insufficient results, 22% of these were caused by poor signal-to-noise or false positive reactions. In general, almost all laboratories could detect MLA in the malignant melanoma, tissue core no. 5, 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 90% (345 of 384) of the laboratories (see Table 1b-c). Used as concentrated format within laboratory developed (LD) assays, 37% (23/62) of the protocols were assessed as sufficient (optimal or good). However, optimal results were only obtained by 3 laboratories. All used the product in a high concentration (1:25-50), HIER in an alkaline buffer and either on the Dako Autostainer with the FLEX+ detection system, or on the Ventana Benchmark where the one optimal result was produced by using OptiView with amplification (see Table 2 and 4).

Table 4. **Assessment marks for mAb clone A103 used as a concentrate on the four main IHC systems and their detection systems.**

Staining platforms	n	Detection systems	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Dako/Agilent Autostainer[3]	1 4	FLEX HRP magenta FLEX+ DAB	- 2	- 2	1 -	-	- -	- -
Dako/Agilent Omnis	1 10	FLEX+ HRP maganta FLEX+ DAB	-	1 7	- 3	-	- 70%	-
Leica Biosystems Bond[4]	6 10	Bond Refine Red Bond Refine DAB	-	1 1	5 9	-	17% 10%	-
Ventana/Roche BenchMark[5]	15 5 7	OptiView DAB UltraView AP UltraView DAB	1 - -	4 4 -	10 1 7	-	33% 80% -	7% - -
Total	59		3	20	36	-		

1) Proportion of sufficient results (optimal or good). (≥5 assessed protocols).
2) Proportion of Optimal Results (OR).
3) Autostainer Classical, Link 48.
4) Bond III, Prime, Max
5) BenchMark GX, XT, Ultra, Ultra Plus

Table 4 shows that protocols based on the mAb clone A103 as concentrate and applied on the Dako/Agilent Autostainer had the highest pass-rate among the IHC platforms, achieving a 100% pass-rate with the EnVision FLEX+ detection system. Protocols applied on Dako Omnis were also based on the EnVision FLEX+ system, but 3 of 11 protocols failed due to excessive background reaction most likely caused by too high antibody concentration. The mAb clone A103 had the lowest success rate on the Leica Biosystem Bond platforms, though the reason for unsatisfactory performance was unclear. For both Dako/Agilent and Leica Biosystem platforms the protocol settings were based on HIER in an alkaline buffer and concentrations of mAb clone A103 between 1:25-100 with a 3-layer detection system. The choice between DAB or AP chromogen did not affect the pass-rate for these platforms.
On the Ventana/Roche platforms, a difference was noted between the choice of chromogen when using UltraView. All 12 protocols submitted with UltraView as detection system used UltraView with amplification; however, no sufficient protocol was observed when using DAB as the chromogen. When applying UltraView Alkaline Phosphatase Red, a pass rate of 80% was observed. The protocols were similar in central settings; HIER using CC1 for approximately 64 minutes and mAb clone A103 diluted at 1:20-50 for 32-44 minutes. Using OptiView, a 33% pass rate was observed. As mentioned earlier, only one protocol produced an optimal result using OptiView with amplification. In total, seven laboratories submitted protocols using the combination of OptiView and amplification with a pass rate of 57% (4 of 7), providing a slightly higher pass-rate than OptiView without amplification. However, protocols based on amplification (tyramide signal amplification) should be meticulously calibrated to prevent background noise.

The **rmAb clone EP43** has shown consistent and promising results in both previous Runs (40, 56 and 60) and the present run 74. Used within a LD assay, 100% (20 of 20) of the protocols based on this clone produced sufficient staining results of which 85% (17 of 20) were assessed as optimal. As shown in Table 2, 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 in contrast to mAb clone A103 has shown to be very robust and seems to be a reliable marker for demonstration of MLA in malignant melanomas with high diagnostic sensitivity and specificity.
In this assessment, several parameters could be used to obtain an optimal result including HIER in alkaline buffer (e.g. CC1, Ventana/Roche), combined retrieval (HIER in CC1 followed by protease 3, Ventana/Roche), use of both 3-step detection systems (e.g. OptiView, Ventana/Roche) or 2-step detection systems (e.g. UltraView, Ventana/Roche) as long as the primary antibody was diluted in the range of 1:25-100.

The **mAb clone BS52** showed a very similar analytical and diagnostic sensitivity and robustness as the rmAb clone EP43, however the protocols submitted and data regarding this clone is still very limited (n=5). Optimal results were observed on 3 of the main IHC systems as shown in Table 2.

With a 100% pass-rate for both EP43 and BS52 it emphasizes the robustness of these antibodies 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 and mAb clone BS52 will not react with these tumors.

In total, 75% (291/384) of the laboratories used a RTU format, which is an increase from 63% (196/312) seen in the previous run 60, 2020.

The most widely used RTU systems for MLA were all based on the **mAb clone A103** from **Ventana/Roche 790-2990**, **Leica Biosystems PA0233/PA0044** and **Dako/Agilent IR/IS633** systems. The overall pass-rate across all three products was notably low. Out of the 274 laboratories that submitted protocols, only two achieved optimal results. The highest proportion of sufficient results were within laboratory developed assays with the Dako/Agilent IR/IS633 product.

A total of 75 laboratories employed the **Dako/Agilent IR/IS633** on non-intended platforms, with the majority using the Dako Omnis (n=68). According to results from the LD assays, the mAb clone A103 requires highly sensitive protocol settings, including a 3-step polymer detection system such as Envision FLEX+ for optimal performance. The pass rate was 20% (10 of 49) when using the 2-layer detection system (EnVision FLEX) on both the Dako Autostainer and Omnis, while the pass rate increased to 87% (35 of 40) when using the EnVision FLEX+ system. These observations were also made in the previous run 60.

The **mAb clone A103 Ventana/Roche 790-2990** was used by a total of 138 laboratories with a pass rate of 12% (17 of 138). The main cause of insufficient results was due to a low analytical and diagnostic sensitivity producing a too weak staining of the neoplastic cells in tissue core no. 4 (see Figs 5-6). This problem was observed with both OptiView and UltraView Universal Alkaline Phosphatase Red detection system (UltraView Red). A total of 17 laboratories achieved sufficient results using HIER in alkaline buffer CC1 for 56-76 minutes, with antibody incubation times ranging from 32-60 minutes. Among these, 10 laboratories employed either UltraView Red or OptiView with amplification.

The **mAb clone A103 Leica Biosystems PA0233/PA0044** had a low pass rate with a total of 5% (2 of 41) applying both VRPS and LMPS. Even though the product was applied accordingly to the vendor recommended protocol and with a 3-layer detection system, the assays with both DAB and Red chromogens had difficulties achieving the expected and required diagnostic sensitivity to stain the neoplastic cells in tissue core no. 4. In the previous Run 60 this system provided the highest pass rate among the RTU systems using the A103 clone.

This was the 10th NordiQC assessment of MLA. A significant and radical decrease in the pass rate was observed (see Graph 1) from 88% in Run 60 (2020) to 34% in Run 74 (2025). In Run 60 the main cause of increase in pass rate from previous runs was primarily related to the change of intended purpose of the assessment focusing on only melanomas and not on steroid producing tumours. In this context it also has to be mentioned that a pass rate can and will be affected by the choice and expression levels of the target analyte in the materials circulated to the participants. In recent assessment runs NordiQC has used the clones **EP43** and **BS52** to select candidate tissues to be included in the proficiency material circulated. In both run 60 and in this present run 74, all the four melanomas included have shown an extensive positive staining reaction in virtually all neoplastic cells with an intensity ranging from moderate to strong. As such it is with great surprise that the distance from the expected result obtained in reference laboratories and reproduced 100% by participants using same settings is so radical to laboratories using less robust assays based on the mAb clone A103. This both related to LD assays and RTU systems based on mAb clone A103 Conclusive, in this Run 74, the mAb clone A103 was the most popular clone used both as a concentrate and as a RTU product. However, this clone had difficulties demonstrating MLA in the more challenging tissue core no. 4 irrespectively of the assay applied. Since the NordiQC run 56 for MLA new clones have been commercially available with more promising and reproducible results especially when staining melanomas with low-level MLA expression as tissue core no. 4

Internal studies performed at NordiQC including a large number of both melanomas and non-melanomas have demonstrated a quite high and significant difference in the diagnostic sensitivity for melanomas and associated H-scores when comparing LD IHC assays based on the rmAb clone EP43 and mAb clone BS52 to a widely used RTU IHC assay for MLA applied within VRPS.
A total of 71 melanomas and 36-non-melanomas were analyzed concerning the diagnostic sensitivity and specificity using an H-score of ≥50 and results was as listed below.

Table 5. **Diagnostic sensitivity and specificity for MLA by IHC clones EP43, BS52 and A103**

	rmAb EP43, LD	**mAb BS52, LD**	**mAb A103, RTU VRPS**
Melanomas, NOS	94% (67/71)	93% (66/71)	82% (58/71)
Non-melanomas	0% (0/36)	0% (0/36)	0% (0/36)

The mean H-scores in the melanomas were 236, 230 and 154 (median 260, 260 and 160) for clones EP43, BS52 and A103, respectively. Conclusive, clone A103 showed a reduced diagnostic sensitivity for melanomas but also displayed a weaker intensity and proportion of positive cells compared to the two other clones, which induces the risk of misdiagnosis.

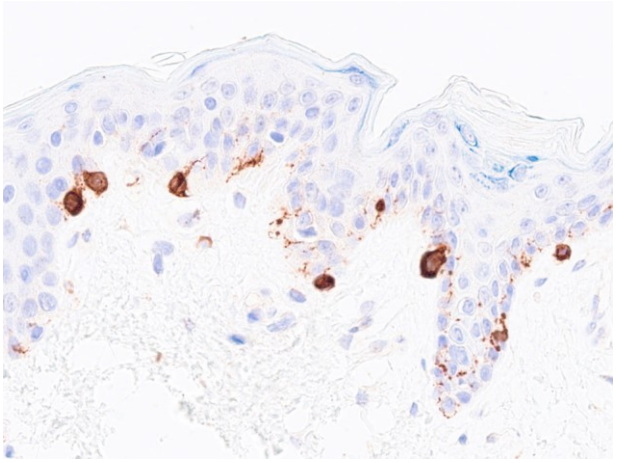


Fig. 1a (x400)
Optimal staining reaction for MLA of the skin using the mAb clone BS52 as a concentrate (1:200), efficient HIER in an alkaline buffer (TRS High, Dako/Agilent) and a 3-step polymer based detection system (EnVision Flex+, GV800/GV823/GV900, Dako/Agilent) - same protocol used in Figs. 2a - 4a. Virtually all melanocytes show a strong cytoplasmic staining reaction and melanocytic dendrites are weak to moderately labelled.

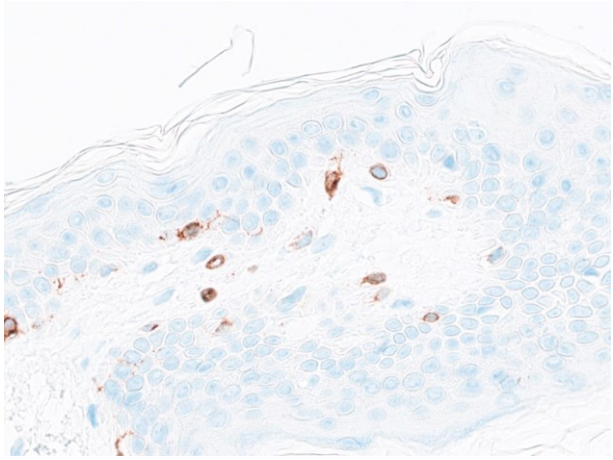


Fig. 1b (x400)
Insufficient staining reaction for MLA of the skin using the mAb clone A103 as RTU product 790-2990, Ventana/Roche as a laboratory developed assay, with efficient HIER time in an alkaline buffer (CC1, Ventana/Roche) for 48 min. and a primary Ab incubation for 56 min. and OptiView as the detection system - same protocol used in Figs. 2b – 4b. Only dispersed melanocytes are demonstrated, the intensity is significantly reduced, and melanocytic dendrites are mostly negative. Compare with Fig. 1a.- same field.

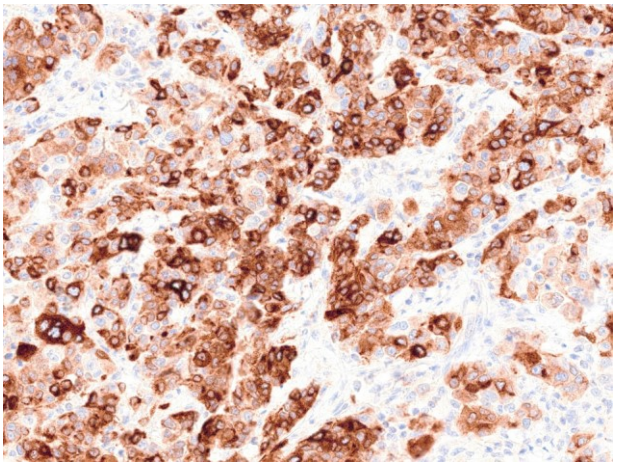


Fig. 2a (x200)
Optimal MLA staining reaction of the malignant melanoma, tissue core no. 4 (low-level expressor), using same protocol as in Figs. 1a - 4a. Virtually all neoplastic cells display a weak to strong cytoplasmic staining reaction.

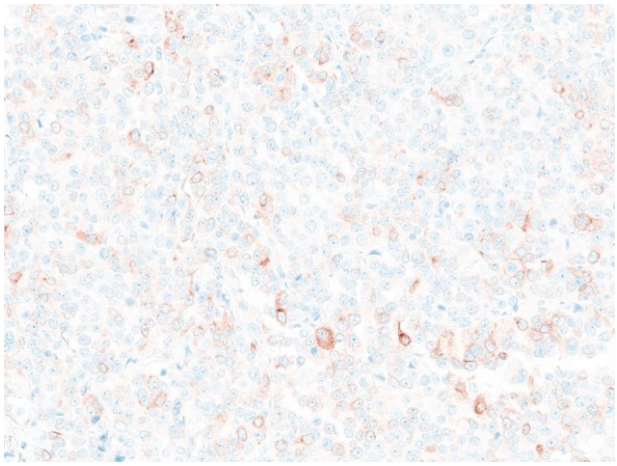


Fig. 2b (x200)
Insufficient MLA staining reaction of the malignant melanoma, tissue core no. 4 (low-level expressor), using the same protocol as in Figs. 1b – 4b. The majority of the neoplastic cells are false negative or only display a weak to moderate cytoplasmic staining reaction. Compare to Fig.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

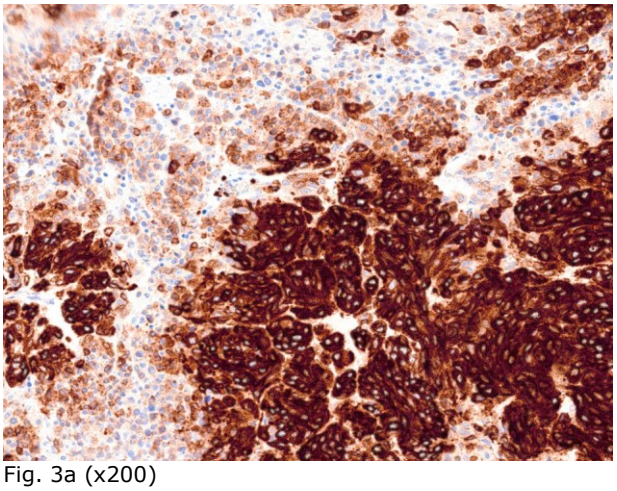


Fig. 3a (x200)
Optimal MLA staining reaction of the malignant melanoma, tissue core no. 5 (high-level expressor), using same protocol as in Figs. 1a – 4a. All neoplastic cells show a strong cytoplasmic staining reaction.

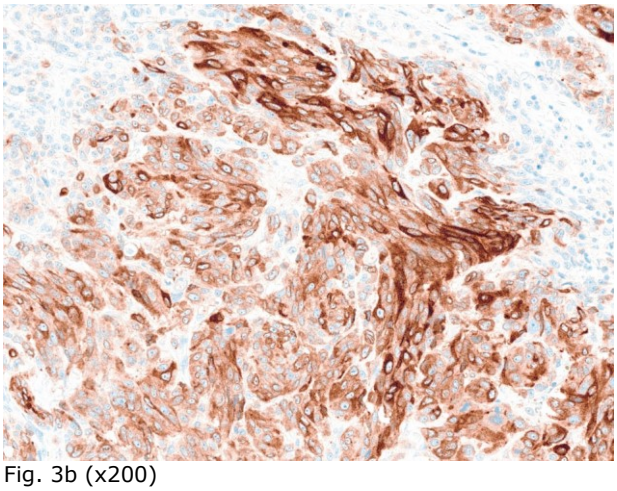


Fig. 3b (x200)
MLA staining reaction of the malignant melanoma, tissue core no. 5 (high-level expressor), using the same insufficient protocol as in Figs. 1b – 4b. The majority of the neoplastic cells show a weak-strong staining reaction. However, compared to other materials of the TMA, the protocol settings gave a too low analytical sensitivity which could lead to a false negative diagnosis.

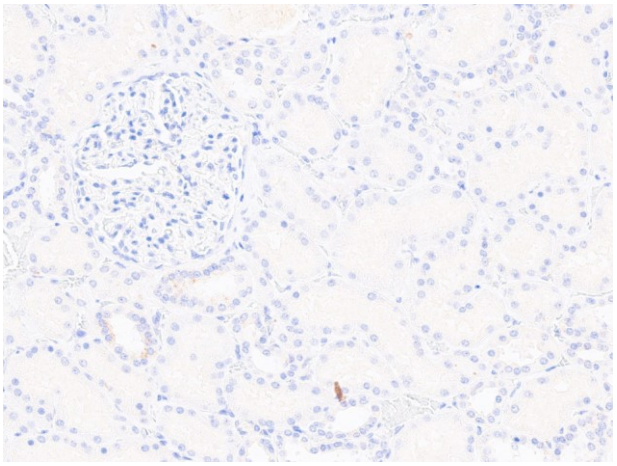


Fig. 4a (x200)
Optimal MLA staining reaction of the kidney using same protocol as in Figs. 1a – 3a. The epithelial cells of tubules display the expected negative staining reaction and the analytical specificity is preserved despite increased sensitivity.

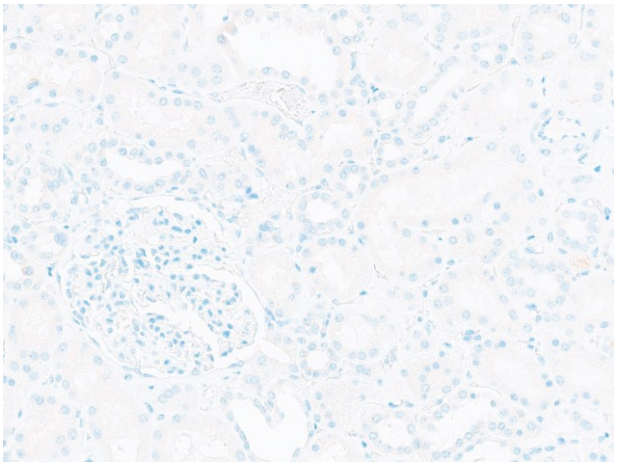


Fig. 4b (x200)
MLA staining reaction of the kidney using same protocol as in Figs. 1b – 3b. Although the tubules show the expected staining pattern, the protocol applied is generally unreliable due to protocol settings giving a low analytical sensitivity, risking misdiagnosis of melanomas – compare Figs.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

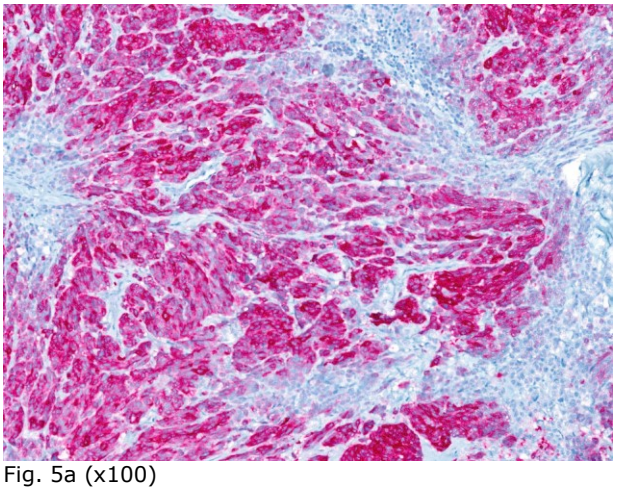


Fig. 5a (x100)
Sufficient (scored good) MLA staining reaction of the malignant melanoma, tissue core no. 5 (high-level expressor), the mAb clone A103 as RTU product 790-2990, Ventana/Roche as a laboratory developed assay, with efficient HIER time in an alkaline buffer (CC1, Ventana/Roche) for 76 min. and a primary Ab incubation for 32 min. and UltraView Red with amplification as the detection system – same protocol used in Fig. 6a.
All neoplastic cells show a strong cytoplasmic staining reaction.

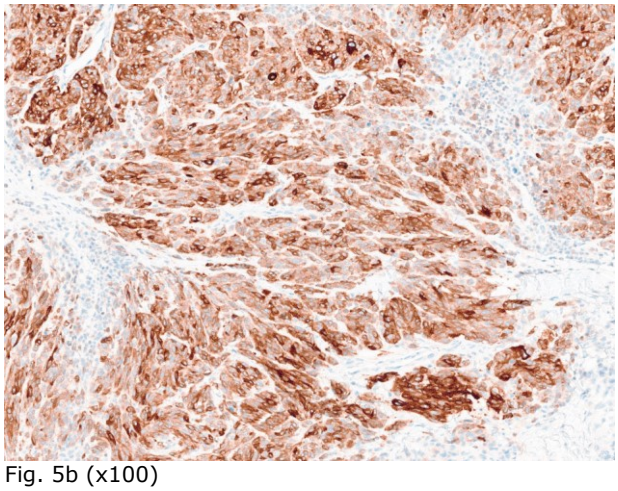


Fig. 5b (x100)
MLA staining reaction of the malignant melanoma, tissue core no. 5 (high-level expressor), the mAb clone A103 as RTU product 790-2990, Ventana/Roche as a laboratory developed assay, with efficient HIER time in an alkaline buffer (CC1, Ventana/Roche) for 76 min. and a primary Ab incubation for 32 min. and UltraView DAB with amplification as the detection system. Same protocol used in Fig 6b and same setup as Figs. 5a – 6a, but with DAB as chromogen. All neoplastic cells show a moderate to strong cytoplasmic staining reaction.

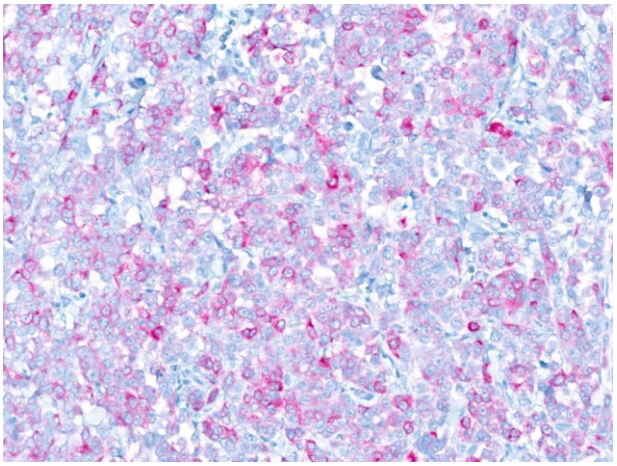


Fig. 6a (x200)
Sufficient (scored good) MLA staining of the malignant melanoma, tissue core no. 4 (low-level expressor), using same protocol as in Fig. 5a. Approximately 50% of the neoplastic cells display a weak to moderate cytoplasmic staining reaction.
See optimal result in Fig. 2a.

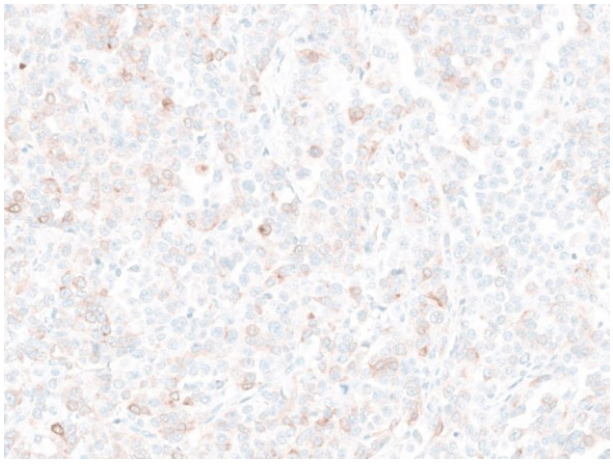


Fig. 6b (x200)
Insufficient (scored borderline) MLA staining reaction of the malignant melanoma, tissue core no. 4 (low-level expressor), using same protocol as in Fig. 5b. The majority of the neoplastic cells are false negative or only display a weak to moderate cytoplasmic staining reaction.
See optimal result in Fig. 2a.

TJ/LE/SN 01.07.25

Version	Description of change and reason	Date	Authorized by
2	Table 1c updated as error found in data entry	10.07.2025	LE/SN
3	Table 1b updated as error found in footnote	11.07.2025	HLK/SN