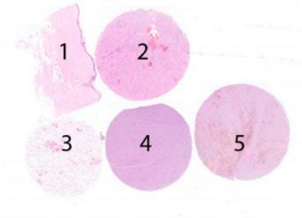
Assessment Run 40 2014

Melanosoma Specific Antigen (MSA)

Material

The slide to be stained for MSA comprised:



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

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MSA staining as optimal were:

- A moderate to strong cytoplasmic staining reaction of the vast majority of neoplastic cells of the two malignant melanomas.
- An at least weak to moderate cytoplasmic staining reaction of the majority of neoplastic cells of the angiomyolipoma.
- No staining reaction in the skin with exception of scattered reactive melanocytes showing a weak to moderate cytoplasmic staining reaction*
- No staining reaction in the kidney*

* Frequently a weak to moderate focal background staining was seen. This staining was fully accepted as this most likely was caused by antigen/chromogen diffusion from the malignant melanomas

Results

187 laboratories participated in this assessment. 29 laboratories used inappropriate antibodies (Abs) (pan-melanoma cocktail or Ab against actins, e.g. mAb clones HHF35 or 1A4). Of the remaining 158 laboratories, 144 (91%) achieved a sufficient mark (optimal or good). Abs used and assessment marks are summarized in table 1 (Page 2).

The most frequent causes of insufficient staining reactions were:

- Omission of HIER
- Too low concentration of the primary antibody
- Usage of biotin based detection systems

Performance history

This was the 3rd NordiQC assessment of MSA. Similar pass rates have been obtained in the two latest runs as seen in table2.

Table 2. **Proportion of sufficient results for MSA in three NordiQC runs performed**

	Run 7 2003	Run 20 2007	Run 40 2014
Participants, n=	66	99	158
Sufficient results	74%	97%	91%

Conclusion

In this run and in concordance with the previous NordiQC assessments for MSA, the mAb clone HMB-45 is a robust, sensitive and specific antibody for detection for MSA. The mAb clone HMB-45 applied as a concentrate could provide optimal result on the 3 main IHC systems (Ventana, Dako and Leica).
The Ready-To-Use (RTU) system from Dako gave the highest proportion of sufficient and optimal results.
Protocols based on HIER and a non-biotin based detection systems gave the highest proportion of optimal results.
Blue nevus or angiomyolipoma is recommended as positive tissue control, in which the majority of neoplastic cells must show an at least weak to moderate and distinct cytoplasmic staining reaction.
Kidney or appendix is recommended as negative tissue control; no staining in the epithelial cells should be seen.

Table 1. **Antibodies and assessment marks for MSA, run 40**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **HMB-45**	71 8 6 2 1 1 1 1 1 1	Dako Leica/Novocastra Thermo/NeoMarkers Genemed Cell Marque DCS ENZO Life Sciences Immunologic Zytomed Unknown	53	33	7	0	93%	93%
Ready-To-Use antibodies								
mAb clone **HMB-45 PM057**	1	Biocare	0	0	1	0	-	-
mAb clone **HMB-45 282M-98**	1	Cell Marque	0	1	0	0	-	-
mAb clone **HMB-45 IS052/IR052**	31	Dako	25	6	0	0	100%	100%
mAb clone **HMB-45 PA0027**	2	Leica	0	2	0	0	-	-
mAb clones **HMB-45 MON-RTU1147**	1	Monosan	0	0	1	0	-	-
mAb clone **HMB-45 MAD-000375QD**	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone **HMB-45 790-4366**	28	Ventana	16	7	5	0	82%	100%
Total	158		95	49	14	0	-	
Proportion			60%	31%	9%	-	91%	

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

Detailed analysis of Melanoma Specific Antigen (MSA), Run 40

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

Concentrated antibodies

mAb clone **HMB-45**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (6/9)*, TRS pH 9 (Dako) (4/8), TRS low pH 6.1, 3-in-1 (Dako) (2/2), Cell Conditioning 1 (CC1; Ventana) (18/41), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (9/12), BERS 1 (Leica) (1/1), Diva Decloaker pH 6.2 (Biocare) (1/2), Tris-EDTA/EGTA pH 9 (8/8) or Citrate pH 6 (3/6) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 81 of 87 (91%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

Table 3. **Proportion of optimal results for MSA using the mAb clone HMB-45 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 **HMB-45**	10/14** (71%)	2/2	17/37 (46%)	-	9/12 (75%)	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 **HMB-45**, product no. IS/IR052, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min. at 95-98°C) and 20 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 30 of 30 (100%) laboratories produced a sufficient staining (optimal or good).

mAb clone **HMB-45**, product no. 790-4366, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were all based on HIER using mild or standard Cell Conditioning 1, 8-36 min. incubation of the primary Ab and UltraView (760-500, +/- amplification kit) or OptiView (760-700) as detection system. Using these protocol settings 17 of 17 (100 %) laboratories produced a sufficient staining.

In this NordiQC assessment of MSA, the prominent feature of an insufficient staining was a generally too weak or false negative staining reaction of structures expected to be demonstrated. Virtually all laboratories were able to demonstrate MSA in neoplastic cells of the two malignant melanomas, whereas the demonstration of MSA in the angiomyolipoma was more challenging and required an optimally calibrated protocol.

Only the mAb clone HMB-45 was used for the demonstration of MSA. Applied as concentrate a high proportion of sufficient results were seen and optimal staining results could be obtained on all 3 main IHC systems from Dako, Ventana and Leica (see table 3). Efficient HIER, usage of a non-biotin based detection system and a titre in the range of 1:20-300 of the primary Ab were the main protocol prerequisites for an optimal result. Efficient HIER seemed to be a central parameter to provide a general high sensitivity in all tissues included in this assessment. Acceptable result in the two melanomas could be obtained by omission of HIER or by using relatively gentle HIER settings (e.g. short efficient HIER time and/or a non-alkaline HIER buffer). However, using these protocol settings, the demonstration of MSA in the angiomyolipoma compromised intensity and reduced proportion of stained cells was seen.

Occasionally an aberrant nuclear staining reaction of neoplastic cells in the two melanomas was seen. This staining pattern was most likely caused by antigen diffusion from the cytoplasmic compartment and was accepted provided that a cytoplasmic staining reaction also was seen.
Antigen/chromogen diffusion from the two melanomas with a high level of MSA expression also caused a focal background staining in the kidney and skin specimens. This phenomenon was not protocol related and thus did not influence the assessment marks.

In this assessment, optimal results could not be obtained if biotin based detection system was used, as the combination of efficient HIER and biotin based detection system resulted in a false positive staining reaction of endogenous biotin in the renal tubules mimicking the specific granular staining reaction of MSA. No significant difference in the proportion of optimal results was seen for 2- and 3-step polymer or multimer based detection systems.

Corresponding RTU systems based on the mAb clone HMB-45 (primarily from Dako and Leica) also provided a high proportion of sufficient and optimal results. The Dako RTU system gave a pass rate of 100% (31 of 31) and 81% were evaluated as optimal. The Ventana RTU system gave a lower proportion of sufficient results of 82% (23 of 28) and 57% were evaluated as optimal. It was observed, that the officially recommended protocol for the Ventana RTU format prod. no. 790-4366 (no epitope retrieval, 8 min. incubation of the primary Ab and UltraView as detection system) was less successful since no optimal results was obtained using these protocol settings. All 16 optimal results was based on modified and laboratory validated protocol settings using HIER in CC1 (mild or standard) and 24-36 min incubation of the primary Ab.

Controls
It is difficult to identify any normal tissue expressing a consistent level of MSA to be used as a recommendable positive tissue control. Normal resting melanocytes in skin are typically not demonstrated by the mAb clone HMB-45. In order to validate a high sensitivity of the protocol an optimal calibration has to be performed. Tumours with both high and low level MSA expression must be used both during technical calibration of the protocol and as positive tissue controls.
In the NordiQC assessments for MSA, blue nevus (run 7, 2003) and angiomyolipoma (run 40, 2014) thus have been superior to malignant melanomas. Using one of these neoplasias, the majority of neoplastic cells must show an at least weak to moderate and distinct granular cytoplasmic staining reaction.
Kidney or appendix can be used as negative tissue controls; no staining reaction should be seen in the epithelial cells.

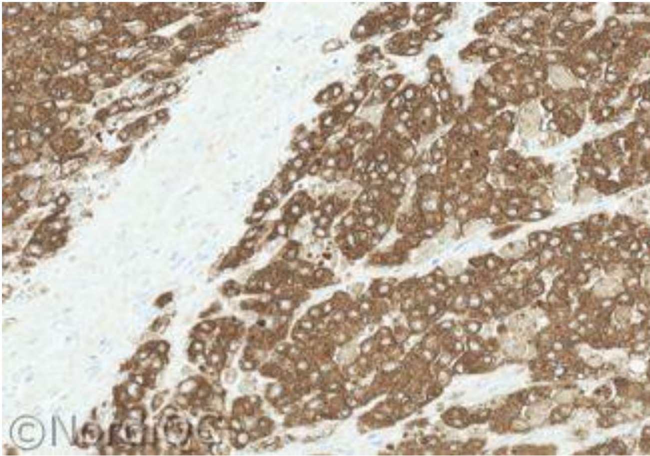


Fig. 1a
Optimal MSA staining of the melanoma tissue core no. 5 in the multi-block using the mAb clone HMB-45 as a concentrate and with HIER. A moderate to strong cytoplasmic staining reaction in virtually all the neoplastic cells is seen. No background staining is seen.
Also compare with Figs. 2a and 3a – same protocol.

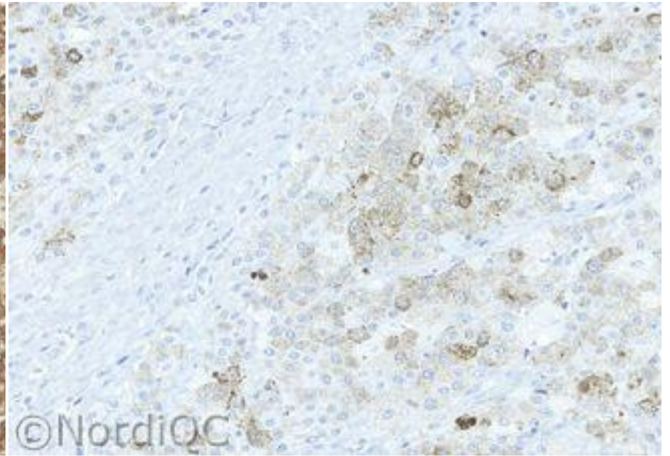


Fig. 1b
Insufficient MSA staining of the melanoma tissue core no. 5 in the multi-block using the mAb clone HMB-45 as a concentrate – same field as in Fig. 1a. The protocol applied provides a too low sensitivity, most likely due to a too low concentration of the primary antibody. Only scattered neoplastic cells are demonstrated. Also compare with Fig. 2b – same protocol.

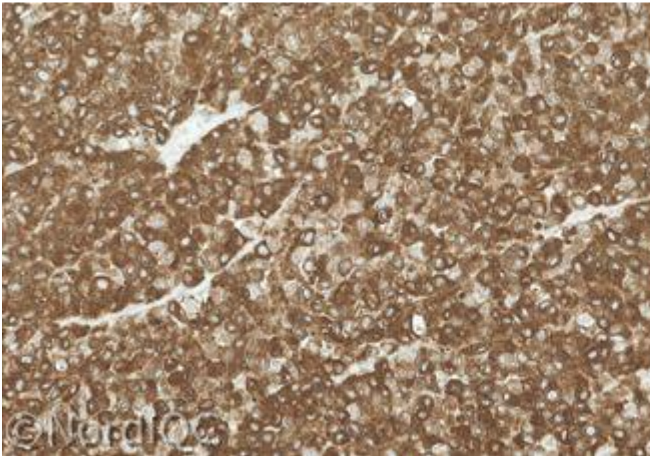


Fig. 2a
Optimal MSA staining of the melanoma tissue core no. 4 in the multi-block using same protocol as in Fig. 1a.
A moderate to strong cytoplasmic staining reaction in virtually all the neoplastic cells is seen. No background staining is seen. Also compare with Fig. 3a – same protocol.

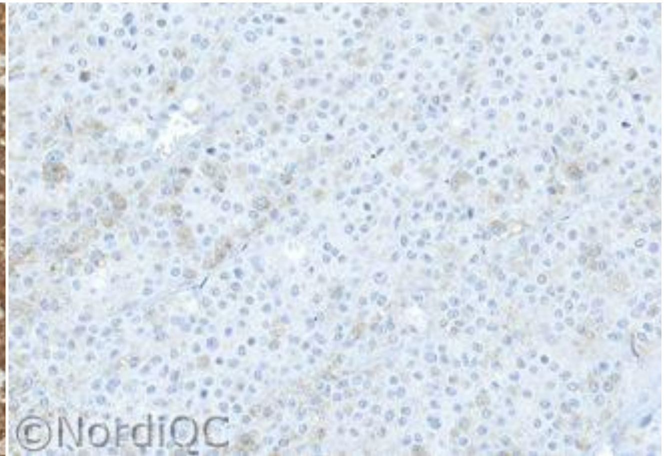


Fig. 2b
Insufficient MSA staining of the melanoma tissue core no. 4 in the multi-block using same protocol as in Fig. 1b – same field as in Fig. 2a. Only a weak and dubious staining reaction of scattered cells is seen.

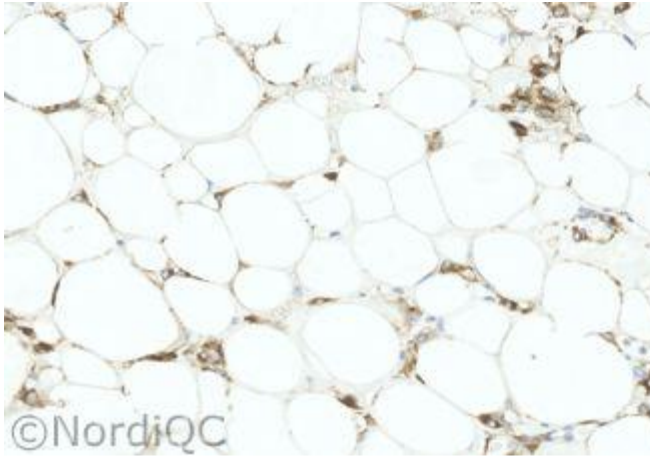


Fig. 3a
Optimal MSA staining of the angiomyolipoma using same protocol as in Figs. 1a and 2b. The vast majority of the neoplastic cells show a weak to moderate and distinct cytoplasmic staining reaction.

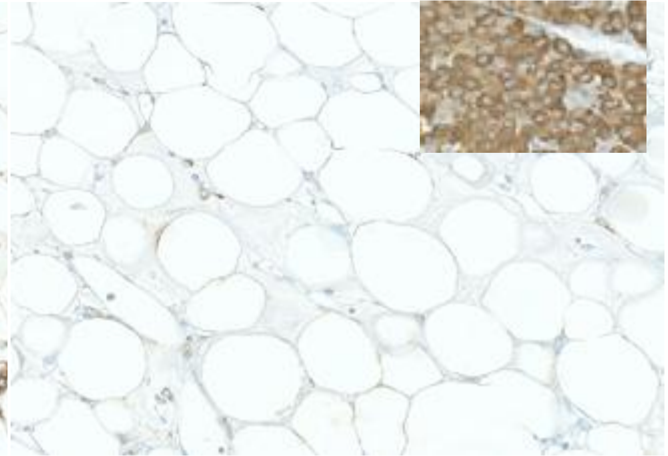


Fig. 3b
Insufficient, false negative MSA staining of the angiomyolipoma - same field as in Fig. 3a. This protocol was based on the mAb clone HMB-45 using the same protocol settings as in Figs. 1a – 3a, except that no HIER was performed. Omission of HIER gives a low sensitivity and especially the demonstration of MSA in the angiomyolipoma is compromised. Note a sufficient staining result in the melanoma was accomplished using same protocol (inset).

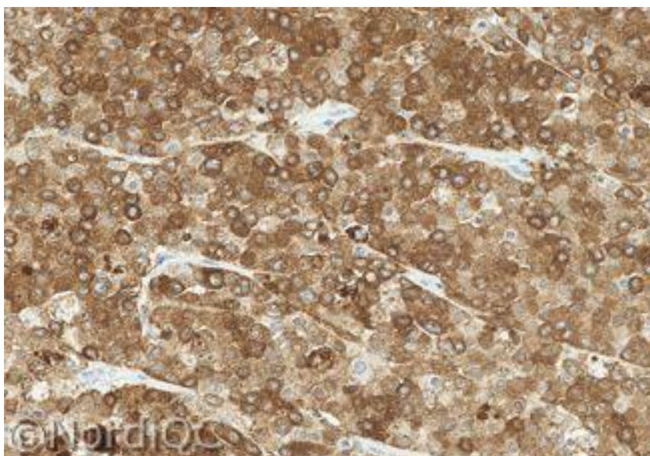


Fig. 4a
Optimal MSA staining of the melanoma tissue core no. 4. A distinct cytoplasmic staining reaction is obtained and no background staining is seen. This protocol was based on HIER in a PT-link using citrate pH 6 as retrieval buffer.

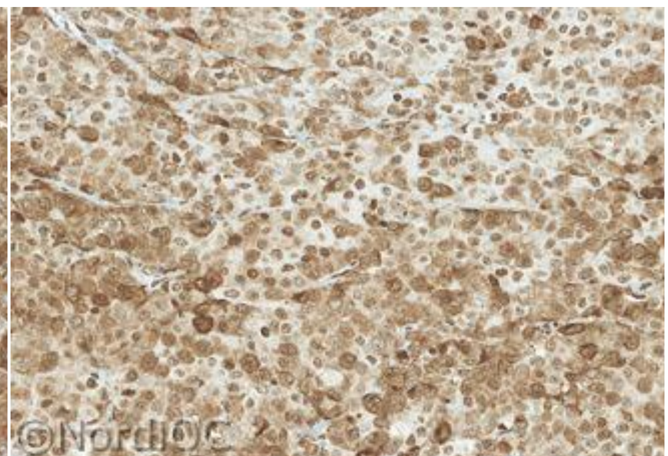


Fig. 4b
Aberrant MSA staining of the melanoma tissue core no. 4. A moderate, predominantly nuclear staining reaction of the neoplastic cells is seen. This staining pattern was frequently observed by using efficient HIER in an alkaline buffer. The overall result was assessed as good, as a coexisting cytoplasmic staining reaction is seen. The staining pattern most likely was caused by diffusion of the MSA antigen from the neoplastic cells with a high level of MSA antigen expression

SN/RR/MV/LE 19-03-2014