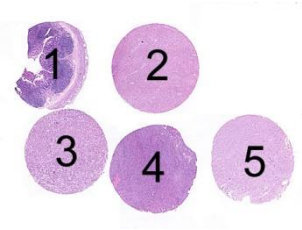
Assessment Run 27 2009

Alpha-smooth muscle actin (ASMA)

The slide to be stained for ASMA comprised:
1. Appendix, 2. Liver, 3 – 4. Leiomyosarcoma, 5. Gastrointestinal stromal tumour (GIST). All tissues were fixed in 10 % neutral buffered formalin.



Criteria for assessing an ASMA staining as optimal included:

- A strong, distinct cytoplasmic staining of all the smooth muscle cells in the muscularis propria, lamina muscularis mucosae and myofibroblasts lining the crypts and surface epithelium of the appendix.
- A moderate to strong, distinct cytoplasmic staining of the majority of the perisinusoidal cells (hepatic stellate cells) in the liver.
- A moderate to strong, distinct cytoplasmic staining of virtually all the neoplastic cells in the leiomyosarcomas and the majority of the neoplastic cells in the GIST.
- A strong, distinct cytoplasmic staining in the smooth muscle cells in virtually all vessels throughout the specimens in the block.

130 laboratories participated in this assessment. 6 used inappropriate antibodies (pan-actin Abs clones HHF35 and HUC1-1). 124 laboratories were assessed, of which 64 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for ASMA, run 27**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1A4**	78 7 6 5 1 1 1	Dako NeoMarkers BioGenex Sigma Biocare Cell Marque Master Diagnostica	26	40	27	6	67 %	86 %
mAb clone **asm-1**	2	Novocastra	0	1	0	1	-	-
pAb **RB-9010-P**	1	NeoMarkers	0	0	0	1	-	-
Ready-To-Use Abs								
mAb clone **1A4, IS611/IR611**	12	Dako	9	2	0	1	92 %	92 %
mAb clone **1A4, 760-2833**	6 1	Ventana Cell Marque	0	1	5	1	14 %	-
mAb clone **1A4, IP001**	1	Biocare	0	0	1	0	-	-
mAb clone **1A4, N1584**	1	Dako	0	0	1	0	-	-
mAb clone **1A4, E046**	1	Linaris	0	1	0	0	-	-
Total	124		35	45	34	10	-	-
Proportion			28 %	36 %	28 %	8 %	64 %	-

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

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **1A4**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) in one of the following buffers: Tris-EDTA/EGTA pH 9 (15/26)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (7/21), Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/8) , EDTA/EGTA pH 8 (1/2) or Citrate pH 6 (1/9). The mAb was typically diluted in the range of 1:50– 1:500 (Dako, M0851) depending on the total sensitivity of the protocol employed. Using the mAb clone 1A4 from Sigma, A2547 the dilution range was 1:8.000

– 20.000. With these protocol settings 49 out of 57 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Ready-To-Use Abs
mAb clone **1A4** (prod. no IS611/IR611, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, 20 min), an incubation time of 20 or 30 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. One lab used the RTU Ab with HIER in MWO and Tris-EDTA/EGTA pH 9 and REAL Envision (Dako) as the detection system. With these protocol settings 11 out of 12 (92 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stainings were:
- Too low concentration of the primary antibody
- Omission of HIER
- HIER in CC1 buffer, Ventana (causing aberrant nuclear staining)
- Less successful RTU mAb clone 1A4 (see table).

In this assessment and in concordance with the observations in the previous ASMA assessments, run 10 and 21, almost all laboratories were able to demonstrate ASMA in the smooth muscle cells in the appendiceal muscle layers and in the large vessels, whereas the prevalent feature of the insufficient staining was a too weak or false negative staining of the perisinusoidal cells in the liver and the neoplastic cells of the leiomyosarcoma (tissue no. 4) and the GIST. This pattern was seen in 28 out of the 43 insufficient results and typically caused by using the mAb clone 1A4 too diluted and/or without HIER.
As also observed in run 21, 2007, an aberrant false positive nuclear staining was seen in e.g., liver cells, lymphocytes and epithelial cells. This pattern was observed in 14 of the 43 insufficient results and was mainly observed, when the mAb clone 1A4 was used with HIER and CC1 on the BenchMark XT, Ventana. The aberrant nuclear reaction was most prominent, when the clone was used relatively concentrated. 28 out of 31 protocols using the mAb clone 1A4 on the BenchMark XT were assessed as insufficient, whereas the remaining 3 protocols were assessed as good. NordiQC is in contact with Ventana to identify the cause.

As recommended in run 21, liver is a recommendable control for ASMA. The perisinusoidal cells in the liver have shown to be a robust critical staining quality indicator, as the majority of the laboratories obtaining an optimal staining could demonstrate ASMA in these cells. It has to be emphasized, that the perisinusoidal cells shall show an as strong as possible reaction without any staining of the liver cell cytoplasm or nuclei (however, a granular reaction in liver cells due to lipofuscin is seen).

This was the third assessment of ASMA in NordiQC and as shown in table 2 the pass rates and proportion of sufficient results are quite constant and still on a relatively low level.

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

	Run 10 2004	Run 21 2007	Run 27 2009
Participants, n=	71	106	124
Sufficient results	62 %	63 %	64 %

In the previous assessments of ASMA (run 10 and 21), a total of 66 laboratories obtaining an insufficient result have been given specific recommendations how to improve their protocol – typically to increase the concentration of the primary Ab and to use HIER. 51 laboratories submitted a new stain in the subsequent run; 26 followed the recommendations, of which 16 improved to good or optimal (62%). 18 laboratories did not follow the recommendations, and 2 of these (11 %) obtained a sufficient staining in the subsequent run. 7 laboratories changed their IHC system completely and 3 of these obtained a sufficient result.

Conclusion
The mAb clone 1A4 is a robust and recommendable Ab for the demonstration of ASMA. HIER is important to obtain an optimal result. Liver is an appropriate control tissue: The majority of the perisinusoidal cells must show a strong, distinct reaction with no cytoplasmic or nuclear staining of the liver cells.

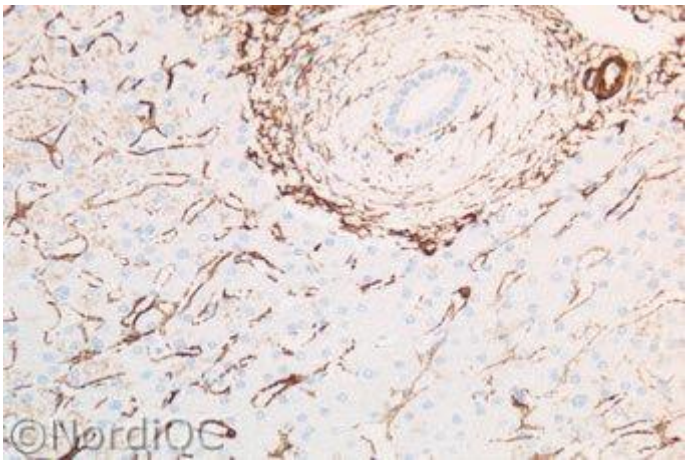


Fig. 1a
Optimal ASMA staining of the liver using the mAb clone 1A4 with HIER. The smooth muscle cells in the portal vessels as well as the perisinusoidal smooth muscle cells show a distinct staining. The liver cells are negative (a weak granular staining is due to lipofuscin).

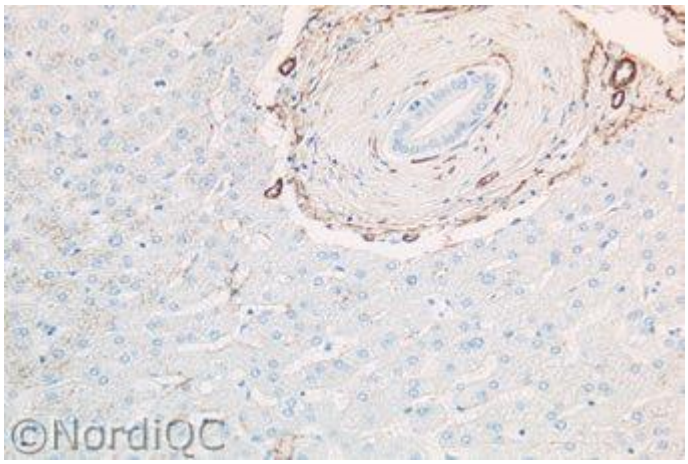


Fig. 1b
Insufficient ASMA staining of the liver using the mAb clone 1A4 in a protocol omitting HIER – same field as in Fig. 1a. The smooth muscle cells in the portal vessels are demonstrated, while the perisinusoidal smooth muscle cells are virtually negative. Also compare with Figs. 2b & 3 b same protocol.

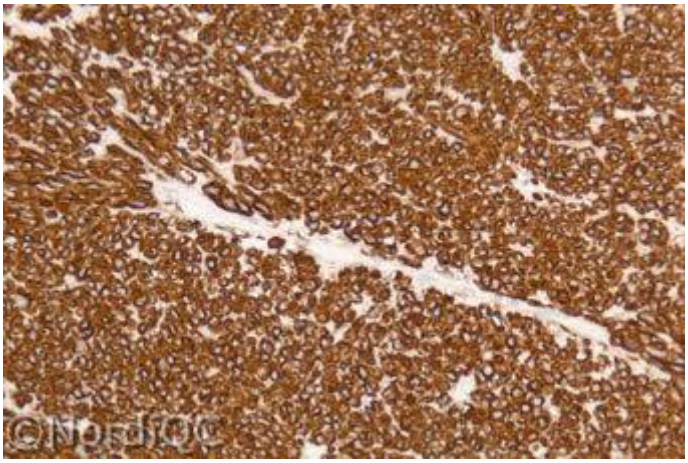


Fig. 2a
Optimal ASMA staining of the leiomyosarcoma tissue no. 3 in the multitissue block using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct reaction with no background reaction.

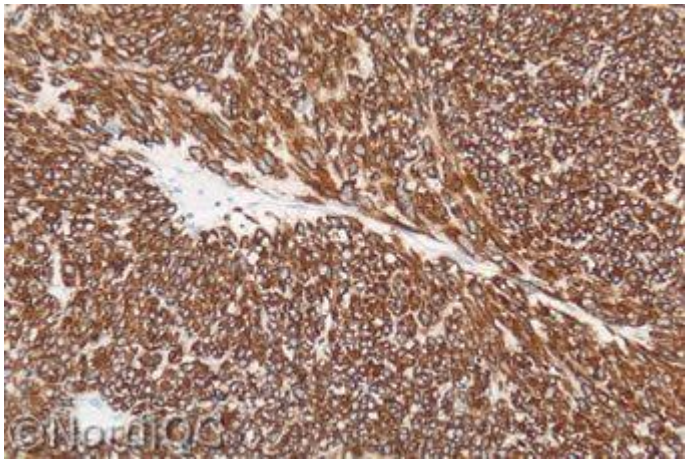


Fig. 2b
ASMA staining of the leiomyosarcoma tissue no. 3 in the multitissue block using same insufficient protocol as in Fig. 1b. Virtually all the neoplastic cells show a strong and distinct reaction with no background reaction – same field as in Fig. 2a. However, also compare with Fig. 3b – same protocol.

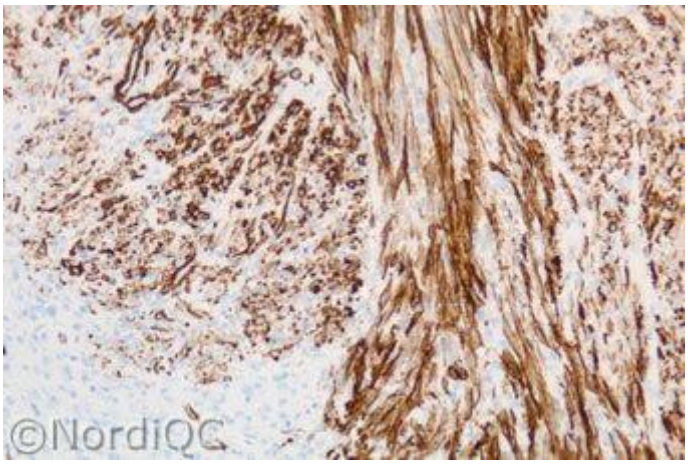


Fig. 3a
Optimal ASMA staining of the leiomyosarcoma tissue no. 4 in the multitissue block using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a moderate to strong and distinct reaction with no background reaction.

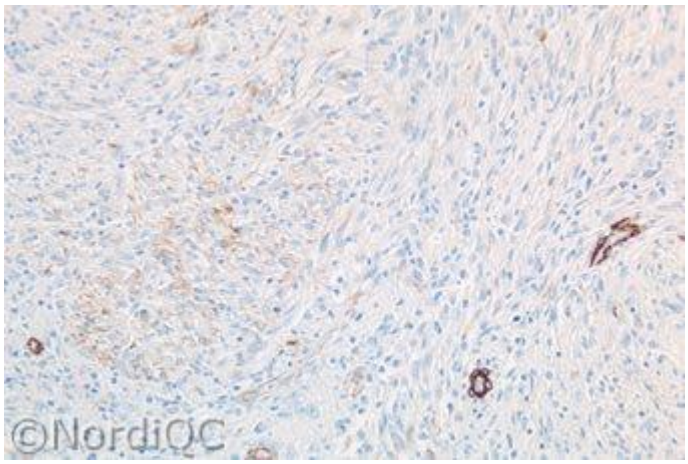


Fig. 3b
Insufficient ASMA staining of the leiomyosarcoma tissue no. 4 in the multitissue block using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak reaction – same field as in Fig. 3a.

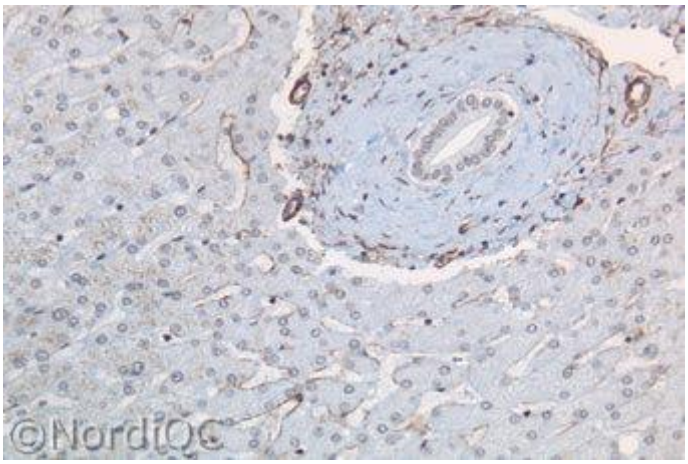


Fig. 4a
Insufficient ASMA staining of the liver using the mAb clone 1A4 with HIER in Cell Conditioning 1 (CC1) on the BenchMark XT, Ventana.
Scattered perisinusoidal smooth muscle cells are demonstrated, but the liver cells and the epithelial cells of the bile duct show a false positive nuclear reaction. This pattern was frequently seen when the mAb clone 1A4 was applied with HIER in CC1 and stained on the BenchMark XT, Ventana.
Compare with Fig. 1a.

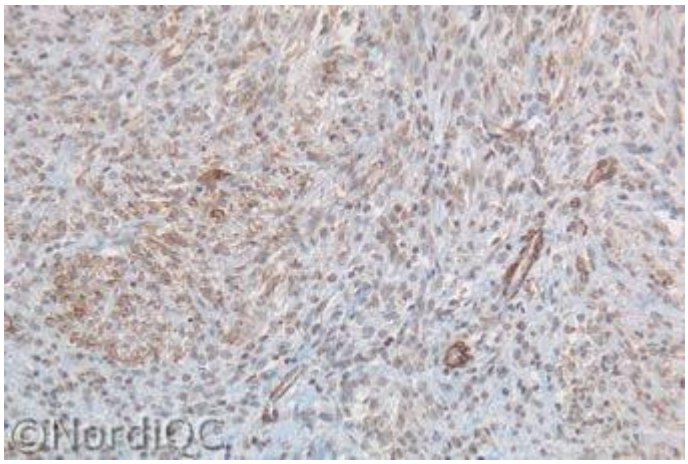


Fig. 4b
Left: Insufficient ASMA staining of the leiomyosarcoma tissue no. 4 in the multi block using same protocol as in Fig. 4a. The neoplastic cells show a false positive positive nuclear reaction, while the specific cytoplasmic reaction is virtually absent.
Compare with Fig. 3a - same field.

SN/HN/MV/LE 4-12-2009