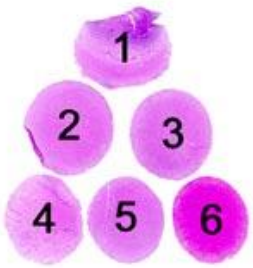
Assessment Run 21 2007

Alpha-smooth muscle actin (ASMA)



The slide to be stained for Alpha-smooth muscle (ASMA) comprised:
1. Appendix, 2. Leiomyosarcoma, 3. Embryonal rhabdomyosarcoma, 4. Normal breast, 5. Gastrointestinal stromal tumour (GIST), 6. Liver.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a ASMA staining as optimal included:

- A strong, distinct cytoplasmic staining of all smooth muscle cells in the muscularis propria, lamina muscularis mucosae and myofibroblasts lining the crypts and surface epithelium.
- A strong, distinct cytoplasmic staining in the myoepithelial cells in the basal layer of the breast glands and ducts.
- A moderate to strong, distinct cytoplasmic staining of the majority of the perisinusoidal cells (hepatic stellate cell, "myofibroblasts") in the liver.
- A strong, distinct cytoplasmic staining of virtually all the neoplastic cells of the leiomyosarcoma and the GIST.
- A strong, distinct cytoplasmic staining of the smooth muscle cells in virtually all vessels throughout the specimens in the multitissue block.

114 laboratories submitted stains. 8 used inappropriate antibodies (pan-actin Abs clone HHF35 and HUC1-1, or h-caldesmon Ab clone h-CALD). 106 laboratories were assessed, of which 32 achieved optimal marks (30 %), 35 good (33 %), 28 borderline (27 %) and 11 poor marks (10 %).

The following Abs were used:
mAb clone **1A4** (Dako, n=75; BioGenex, n=7; Ventana, n=7; Sigma-Aldrich, n=6; NeoMarkers, n=5; BioCare, n=2; Cell Marque, n=1; Oncogene, n=1; Linaris n=1)
mAb clone **ASM-1** (Progen, n=1)

Optimal staining for ASMA in this assessment was obtained with the mAb clone **1A4** (32 out of 105).

1A4: Protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (28/51)*, Bond Epitope Retrieval Solution 2 (Bond, Leica-Microsystems) (2/3), Target Retrieval Solution pH6,1 (TRS, Dako) (1/1) or Citrate pH 6 (1/9) as HIER buffer. The mAb was typically diluted in the range of 1:75 – 1:1.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 50 out of 57 (88 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

All of 7 protocols based on **1A4** as a Ready-To-Use (RTU) Ab gave an insufficient (weak or false negative) staining result, despite the protocol settings being otherwise identical to those based on a concentrated Ab giving sufficient results.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- No or insufficient epitope retrieval (particularly with citrate buffer)
- Less successful RTU mAb clone **1A4.**

In the assessment and in concordance with the observations in the previous ASMA assessment in run 10 2004 almost all laboratories were able to demonstrate ASMA in the smooth muscle cells in the appendiceal muscle layers and the myoepithelial cells of the breast, whereas the prevalent feature of the insufficient staining in this run was a too weak or false negative staining of the perisinusoidal cells, the leiomyosarcoma and the GIST. This pattern was seen in 28 out of the 39 insufficient results and typically caused by using the clone 1A4 too diluted or in the RTU format, or without HIER. A false positive staining of nuclei was observed in 8 of the 39 insufficient results. Efficient HIER combined with a too high concentration of the clone 1A4 seemed to cause this pattern. The perisinusoidal cells in the liver is a robust stain quality indicator, as the majority of the laboratories with

optimal staining of the other tissues 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

This was the second run of ASMA. In the assessment of ASMA in run 10, where 71 laboratories participated, 27 laboratories obtained an insufficient mark. Each was given a specific recommendation to improve their protocol. 20 of them submitted a new ASMA stain in run 21. 13 followed the recommendation, of which 8 improved to good or optimal (62 %). 5 laboratories did not follow the recommendation, and none of these obtained a sufficient staining in run 21.
Two laboratories changed their entire system and can not be taken in account – one improved, the other did not. While the number of participants doubled from run 10 to the current run, the results and observations are comparable.

Conclusion
The mAb clone 1A4 is a robust Ab for the demonstration of ASMA. HIER is important to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated in order to avoid both a false negative and false positive reaction. Liver is an appropriate control tissue: The perisinusoidal cells must show a strong reaction with no staining of the liver cells.

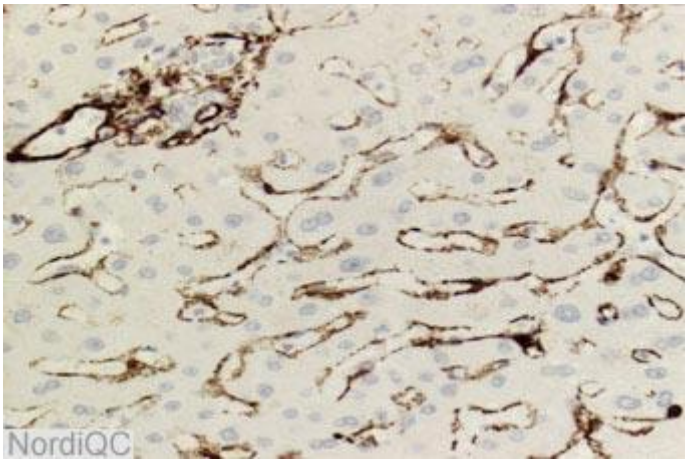


Fig. 1a
Optimal ASMA staining of the liver using the mAb clone 1A4 with HIER. Both the smooth muscle cells of the portal vessels and the perisinusoidal cells show a distinct staining. The liver cells are negative.

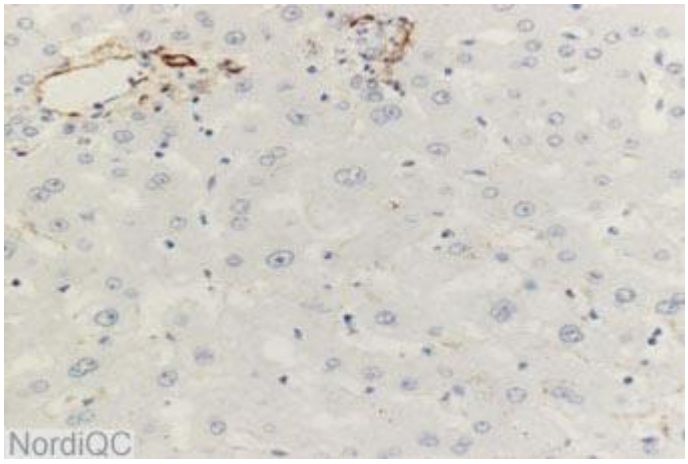


Fig. 1b
Insufficient ASMA staining of the liver using the mAb clone 1A4 in a protocol omitting HIER.
Only the smooth muscle cells of the portal vessels are demonstrated, while the perisinusoidal cells are negative.

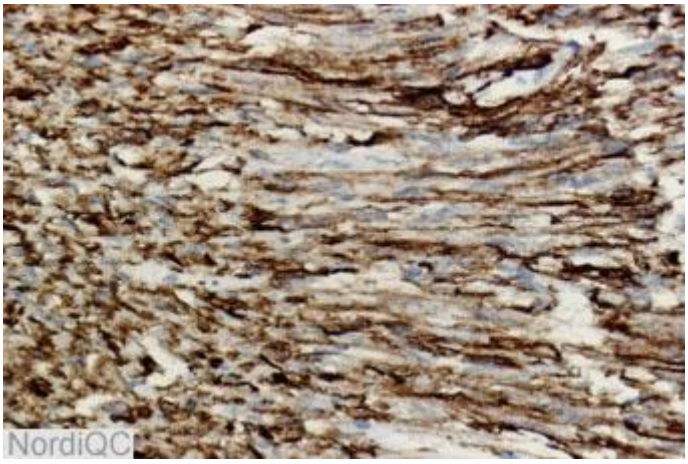


Fig. 2a
Optimal ASMA staining of the GIST using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong distinct reaction with no background reaction.

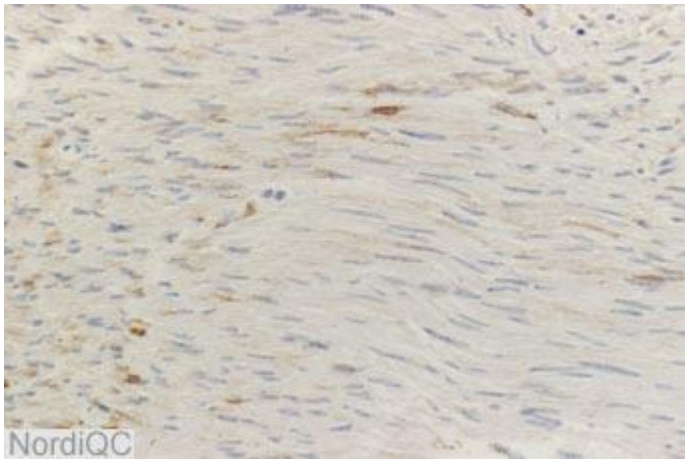


Fig. 2b
Insufficient ASMA staining of the GIST using same protocol as in Fig. 1b. Only scattered neoplastic cells show a weak reaction – same field as in Fig. 2a.

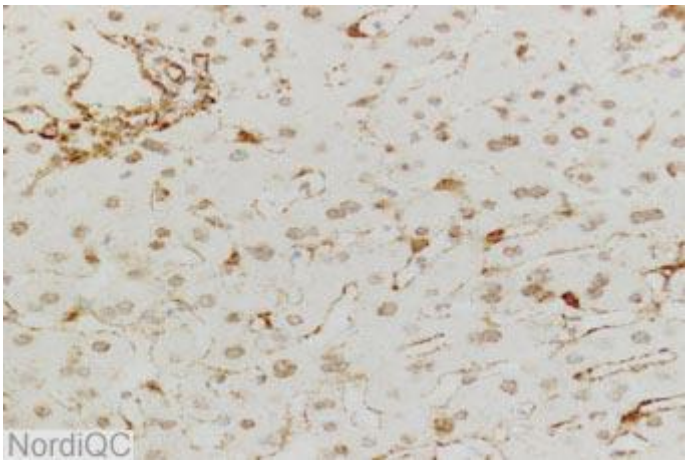


Fig. 3a
Insufficient ASMA staining of the liver using the mAb clone 1A4 – probably too concentrated. The portal smooth muscle cells and the perisinusoidal cells are demonstrated, but at the same time the liver cells show a positive nuclear reaction. Compare with Fig. 1a.

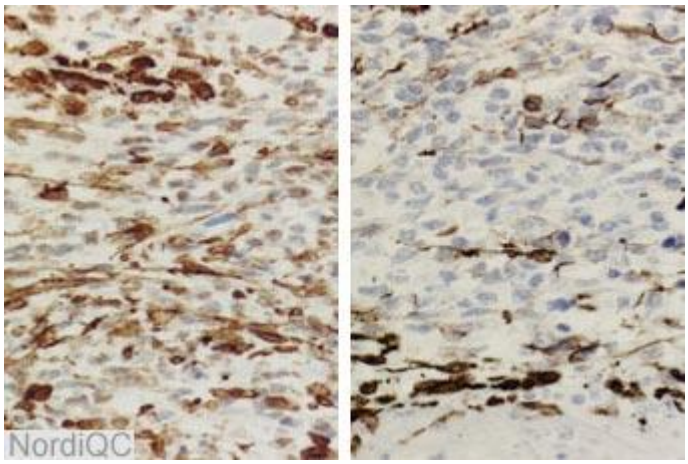


Fig. 3b
Left: Insuffient ASMA staining of the rhabdomyosarcoma using same protocol as in Fig.3a. The neoplastic cells show a positive nuclear reaction.
Right: Optimal ASMA staining of the rhabdomyosarcoma using same protocol as in Fig. 1a & 2a. No nuclear reaction is observed.

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