Assessment Run 10 2004

Alpha-smooth muscle actin (ASMA)

The slide to be stained for alpha-smooth muscle actin (ASMA) comprised:
1. Uterine leiomyosarcoma, 2. Breast fibrocystic disease, 3. Gastric gastro intestinal stromal tumour (GIST), 4. Small intestinal GIST, 5. Appendix.

Criteria for assessing an ASMA staining as optimal included:
A strong and distinct cytoplasmic reaction of the appendiceal smooth muscle cells (vessels and muscular layers) and myofibroblasts, the myoepithelial cells of the glands and ducts of the breast fibrocytic disease and the uterine leiomyosarcoma, and a focal cytoplasmic reaction of the two GISTs.

71 laboratories submitted stainings. At the assessment 30 achieved optimal staining (42 %), 14 good (20 %), 19 borderline (27 %) and 8 poor staining (11 %).

The following appropriate mAbs were used:
clone 1A4 (DakoCytomation, n=55; Sigma, n=4; BioGenex, n=1; BioMakor, n=1; NeoMarkers, n=1)
clone asm1 (Novocastra, n=1; Ventana, n=1)
Seven laboratories used an inappropriate mAb, clone HHF35 (Enzo, n=5; DakoCytomation, n=2), which is a pan-actin Ab.

In this assessment optimal stainings could only be obtained with the mAb clone 1A4. In the optimal protocols all used HIER, 29 with Tris-EDTA/EGTA pH 9 as the heating buffer and one with Target Retrieval Solution pH 6. 1A4 was typically used in a dilution of 1:100–600 (depending on the total sensitivity of the used protocol). Twelve laboratories used the mAb clone 1A4 without pre-treatment and two used proteolytic pre-treatment. None of these protocols resulted in optimal stainings, twelve were assessed as insufficient.

Besides being less specific for ASMA, clone HHF35 revealed a rather poor sensitivity for this actin (no staining was optimal, one was good, the others insufficient).

The majority of laboratories were able to detect ASMA in the appendiceal muscular layers and breast myoepithelial cells. In the insufficient stainings (primarily performed without HIER) the two GIST's were typically negative and the leiomyosarcoma only weakly labelled.

In the periphery of normal germinal centres and lining the surface epithelial cells of the appendix tiny myofibroblasts were identified in the optimal stainings only, indicating that these cells might serve as an appropriate positive control for detection of ASMA.

The most frequent causes of insufficient stainings were:
- Inappropriate choice of primary Ab
- Inappropriate epitope retrieval
- Insufficient HIER, i.e. too short heating time
- Too low concentration of the primary antibody.

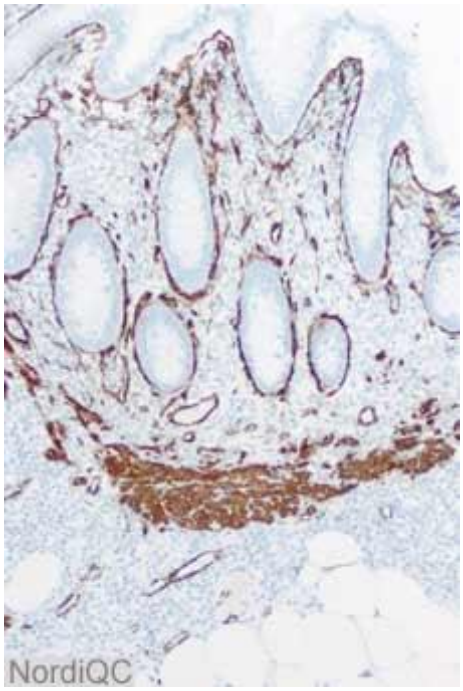

Fig. 1a
Optimal ASMA staining (mAb clone 1A4) of the normal appendix. Intense staining is seen in the smooth muscle cells of the lamina muscularis mucosae. More important the tiny layer of myofibroblasts lining the surface epithelial cells is stained (compare with Fig. 2a).

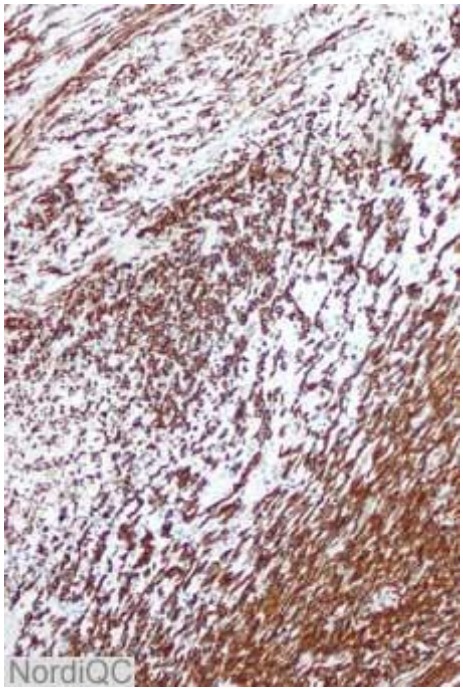

Fig. 1b
Optimal ASMA staining (mAb clone 1A4) of the leiomyosarcoma.
The cells show a strong, distinct cytoplasmic staining (compare with Fig. 2b).

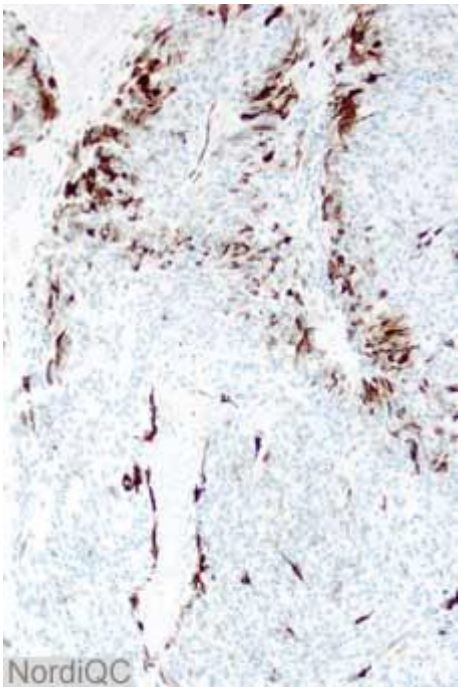

Fig. 1c
Optimal ASMA staining (mAb clone 1A4) of the small intestinal GIST.
Focally the cells show a strong, distinct cytoplasmic staining (compare with Fig. 2c).

Fig. 2a
A strong ASMA staining is seen in the smooth muscle cells of the lamina muscularis mucosae.
However, the tiny layer of myofibroblasts lining the surface epithelial cells is negative (arrow; compare with Fig. 1a).

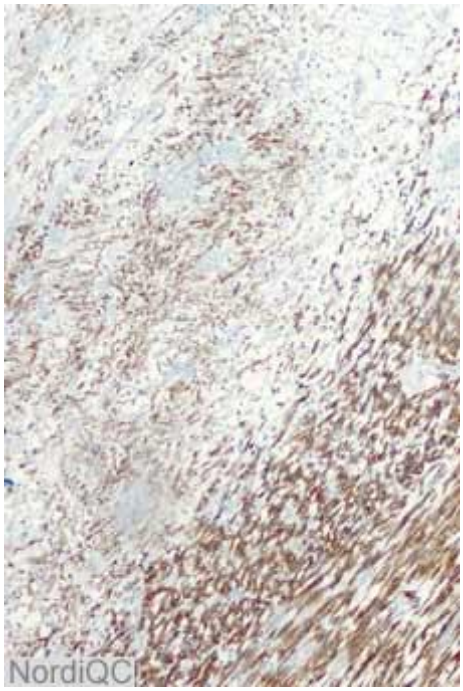

Fig. 2b.
Insufficient ASMA staining (mAb clone 1A4) of the neoplastic cells in the leiomyosarcoma. The cells show a heterogeneous staining (compare with Fig. 1b).

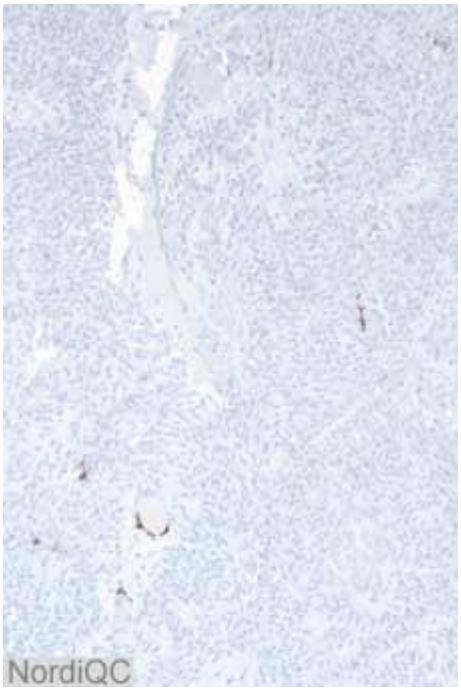

Fig. 2c
Insufficient ASMA staining (mAb clone 1A4) of the neoplastic cells in the small intestinal GIST.
All the neoplastic cells are negative. Only the vascular smooth muscle cells are stained (compare with Fig. 1c).

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