

The slide to be stained for CD31 comprised:

1. Appendix, 2. Haemangiosarcoma, 3. Small intestinal lymphangioma, 4. Liver.

Criteria for assessing a CD31 staining as optimal included: A strong and distinct predominantly membranous but also cytoplasmic staining of normal vascular endothelial cells (including hepatic sinusoidal endothelial cells and lymphatic vessels), the haemangiosarcoma and the small intestinal lymphangioma, and a weaker staining of activated B- and T-cells.



59 laboratories submitted stainings. At the assessment 22 achieved optimal staining (37 %), 17 good (29 %), 12 borderline (20 %) and 8 (14%) poor staining.

The following Abs were used:

mAb clone JC70A (DakoCytomation, n=56; Cell Margue, n=1)

mAb clone 1A10 (Novocastra, n=1; Ventana, n=1)

In this assessment optimal stainings could only be obtained with the mAb clone JC70A.

In the optimal protocols all used HIER, the majority with Tris-EDTA/EGTA pH 9 as the heating buffer (16 out of 27 were optimal), the remainder Citrate pH 6 (1 out of 9 was optimal) or Target Retrieval Solution (DakoCytomation; 3 out of 5 were optimal). None of the 13 laboratories using proteolytic pretreatment obtained an optimal staining, and only one was assessed as good.

mAb JC70A was used in the range of 1:20 – 150 depending on the total sensitivity of the used protocol.

The majority of laboratories with insufficient staining were able to detect CD31 in the endothelial cells of the large vessels in all materials of the multi tissue-block while CD31 in the hemangiosarcoma and the lymphangioma could not be revealed or were only focally demonstrated. In all optimal stainings the hepatic sinusoidal endothelial cells and the activated lymphocytes were demonstrated, while these cells were very weakly stained or negative in the insufficient stainings.

The most frequent causes of insufficient stainings were (often in combination):

- Inappropriate choice of epitope retrieval: Proteolytic pretreatment
- Insufficient epitope retrieval (typically citrate pH 6 as the heating buffer combined with a low sensitivity of the protocol)
- Inappropriate choice of primary Ab
- Too low concentration of the primary Ab

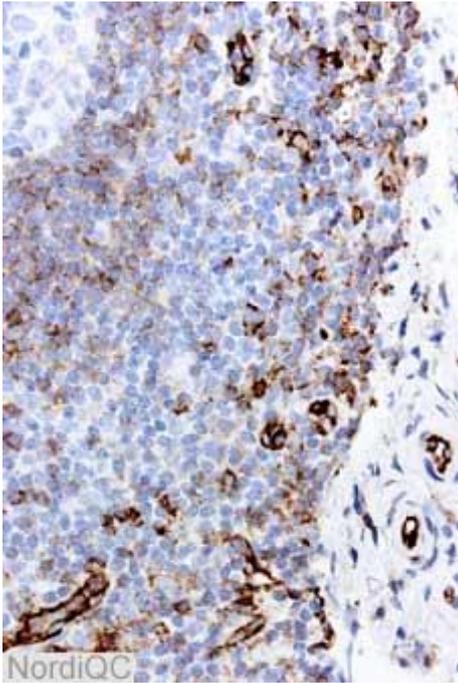


Fig. 1a
Optimal CD31 staining of the appendix. Intense staining is seen in the vascular endothelial cells. The activated lymphocytes are also demonstrated.

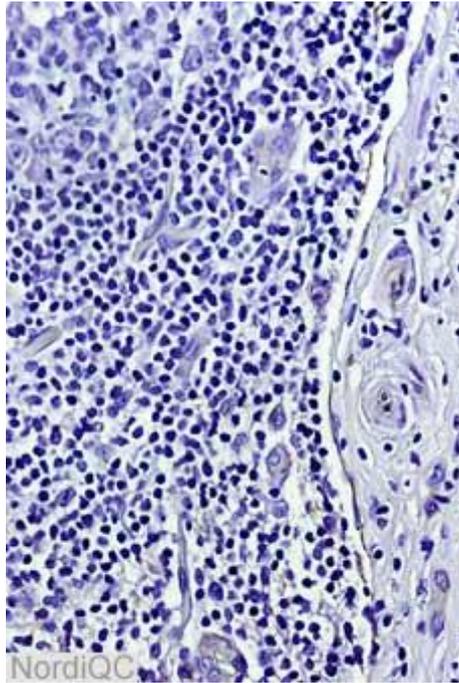


Fig. 1b
Insufficient CD31 staining of the appendix (same field as in Fig. 1a). The endothelial cells are only weakly stained and the lymphocytes are negative.

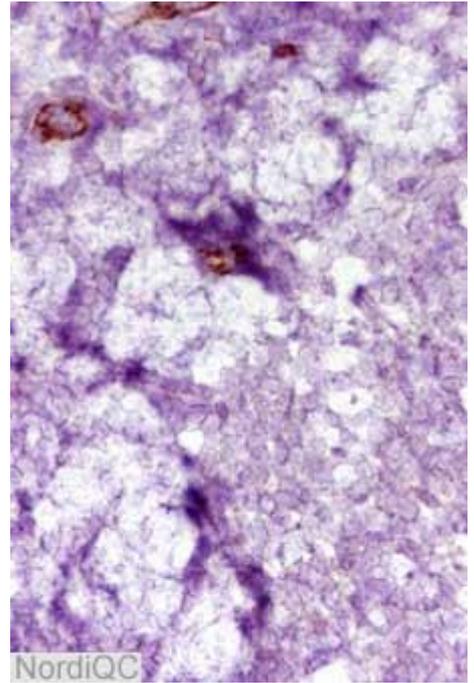


Fig. 1c
Insufficient CD31 staining of the appendix (same field as in Fig. 1a). The endothelial cells are stained, but the morphology is severely impaired due to excessive proteolytic epitope retrieval.

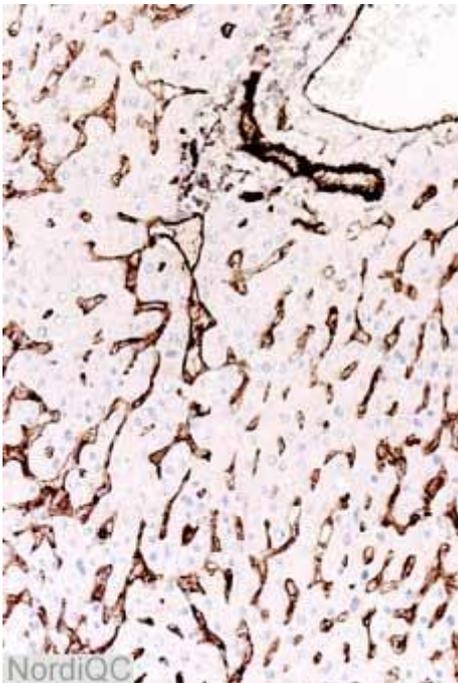


Fig. 2a
Optimal CD31 staining of the liver. Intense staining is seen in the large vessel and hepatic sinusoidal endothelial cells.

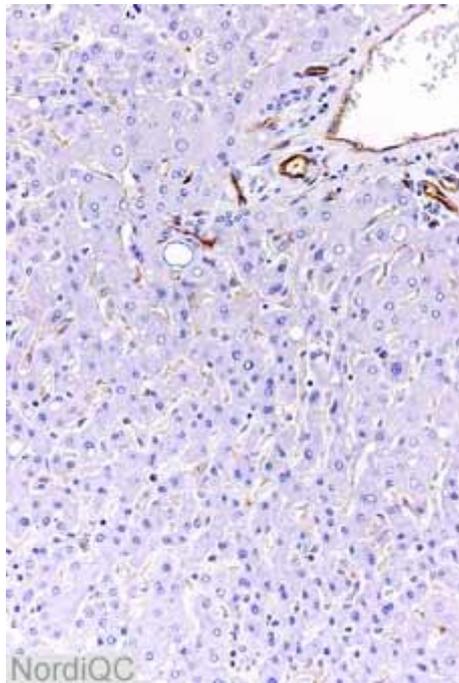


Fig. 2b
Insufficient CD31 staining of the liver. Only the endothelial cells of the large vessels are moderately stained, whereas the hepatic sinusoidal endothelial cells are very weakly stained (same protocol used in Fig. 1b).

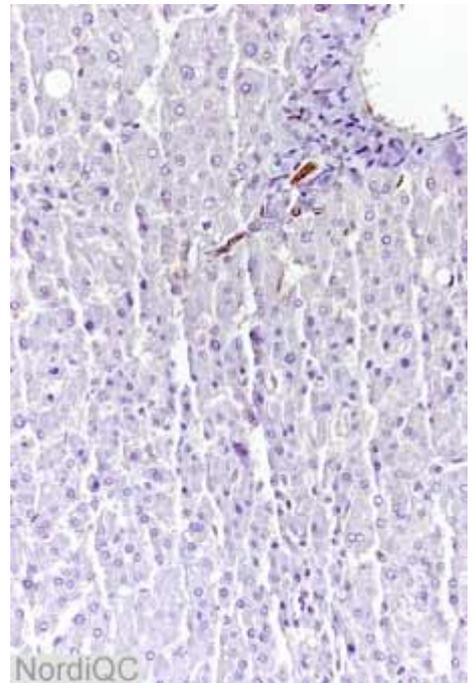


Fig. 2c
Insufficient CD31 staining of the liver. Only the endothelial cells of the large vessels are weakly stained, whereas the hepatic sinusoidal endothelial cells are negative (same protocol used in Fig. 1c).

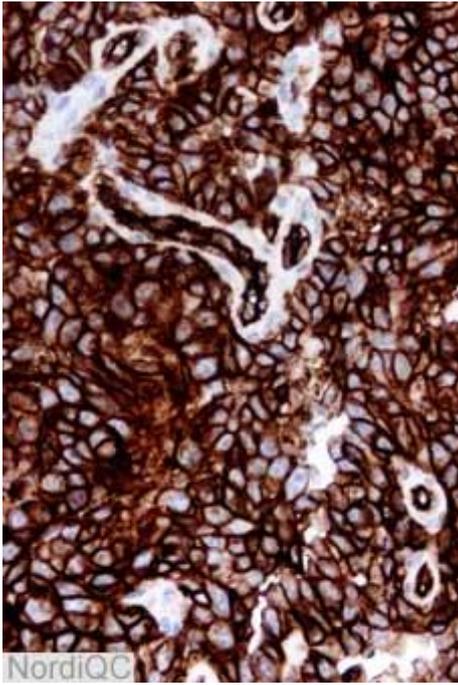


Fig. 3a
Optimal CD31 staining of the haemangiosarcoma. Intense membranous staining is seen in all of the neoplastic cells.

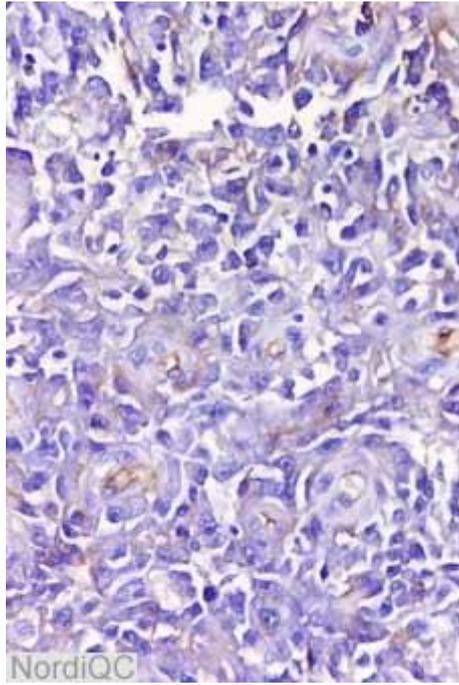


Fig. 3b
Insufficient CD31 staining of the hemangiosarcoma. The endothelial cells of the small vessels are weakly stained, whereas the neoplastic cells in between are negative (same protocol used in Fig. 1b).

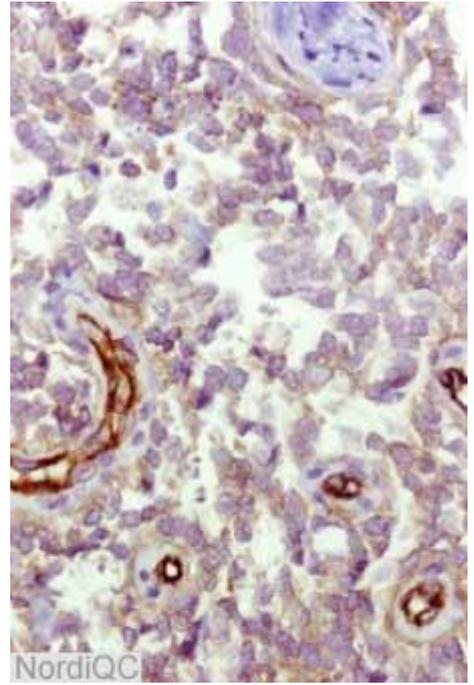


Fig. 3c
Insufficient CD31 staining of the hemangiosarcoma. The endothelial cells of the small vessels are moderately stained, whereas the neoplastic cells in between are weakly and undistinctly stained. The morphology is severely impaired due to excessive retrieval (same protocol used in Fig. 1c)

SN/MV/LE 21-6-2004