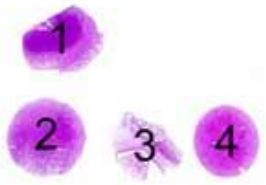
Assessment Run 11 2004

Factor VIII related antigen (FVIII)

The slide to be stained for factor VIII related antigen (FVIII) comprised:
1. Appendix, 2. Haemangiosarcoma, 3. Small intestinal lymphangioma, 4. Liver.



Criteria for assessing a FVIII staining as optimal included: A strong and distinct cytoplasmic staining of vascular endothelial cells of both blood and lymphatic vessels, the hemangiosarcoma and the lymphangioma.

62 laboratories submitted stainings. At the assessment 12 achieved optimal staining (19 %), 14 good (23 %), 18 borderline (29 %) and 18 (29%) poor staining.

The following Abs were used:
pAb A0082 (DakoCytomation, n=49)
pAb 760-2642 (Ventana, n=2)
mAb clone F8/86 (DakoCytomation, n=9; DBS-Bio-Optica, n=1)
mAb clone Z002 (Zymed, n=1)

In this assessment optimal staining could only be obtained with the pAbs A0082 (11 labs. out of 49) and 760-2642 (1 lab. out of 2).
Using A0082 optimal staining could be obtained with proteolysis (8 labs.) and HIER with Tris/EDTA/EGTA as the heating buffer (3 labs.).
In the optimal staining with pAb 760-2642, proteolytic pre-treatment was used.
pAb A0082 was used in the range of 1:200 – 4000 depending on the total sensitivity of the protocol. pAb 760-2642 was a Ready-To-Use Ab.

The majority of laboratories were able to detect FVIII in the endothelial cells of the large vessels in all multi tissue-block specimens. However, in the optimal staining a distinct cytoplasmic, sometimes dot-like demonstration of FVIII in the hemangiosarcoma was seen and in the lymphangioma the endothelial cells were clearly demonstrated. The typical pattern of insufficient staining was a too weak or false negative reaction of these structures primarily due a too dilute primary Ab. conc. of the pAb A0082 (frequently in the range of 1:6.000 – 15.000).
In the appendix and liver the optimal staining demonstrated FVIII in the endothelial cells in arteries, capillaries, liver sinuoids, venules and lymphatic vessels, whereas an insufficient staining typically only demonstrated FVIII in the venules.

The most frequent causes of insufficient stainings were (often in combination):
- Inappropriate choice of primary Ab
- Too low concentration of the primary antibody
- No epitope retrieval

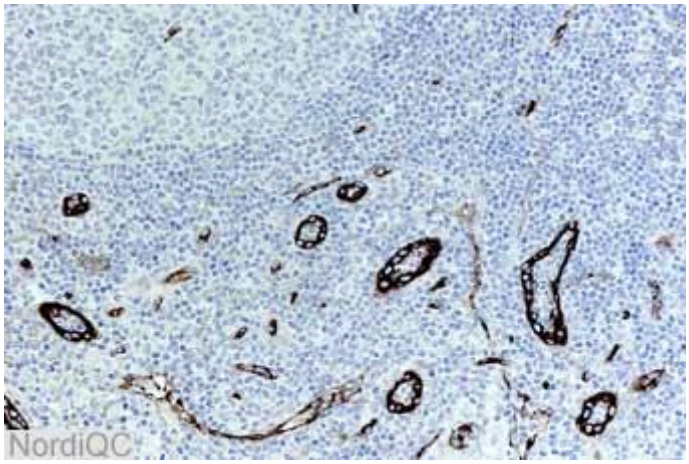


Fig. 1a
Optimal FVIII staining of the appendix. Intense staining is seen in the endothelial cells of all vessels.

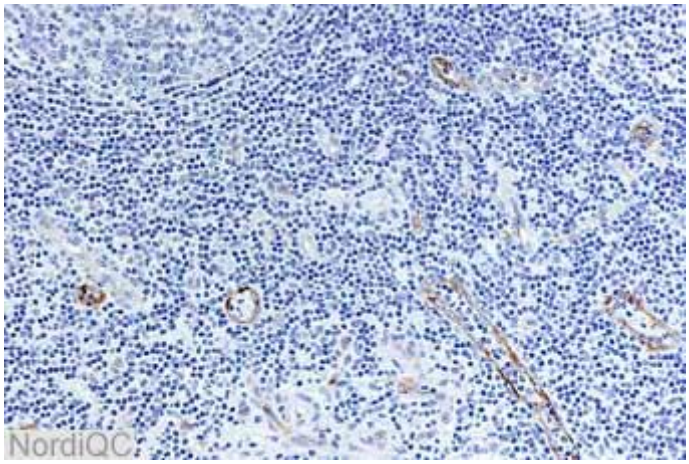


Fig. 1b
Insufficent FVIII staining of the appendix. The endothelial cells of the venules are weakly stained whereas other vessels are unstained.

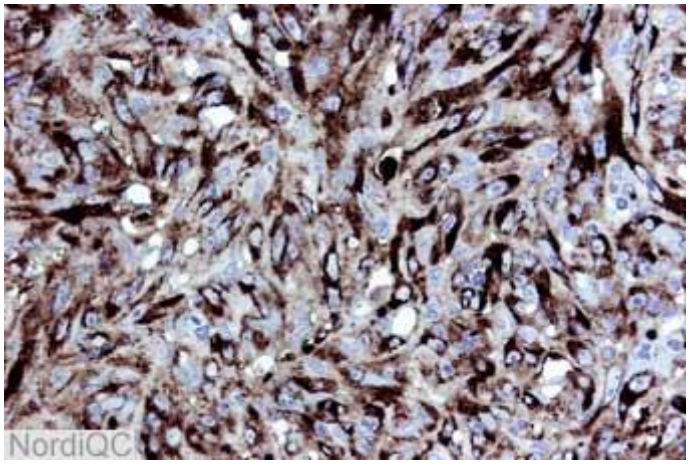


Fig. 2a
Optimal FVIII staining of the hemangiosarcoma. Intense cytoplasmic staining is seen in the majority of the neoplastic cells.

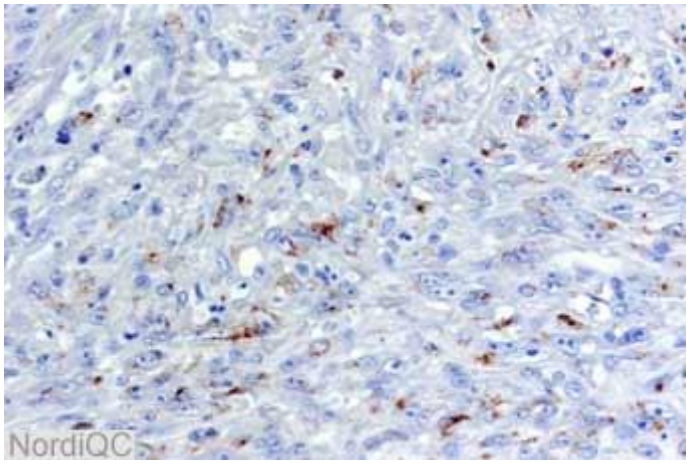


Fig. 2b
Insufficient FVIII staining of the hemangiosarcoma (same protocol as in Fig 1b). The neoplastic cells are only weakly stained (compare with Fig. 2a)

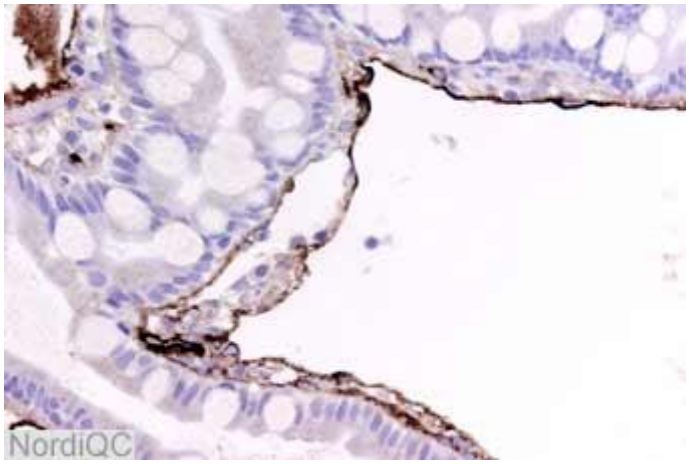


Fig. 3a
Optimal FVIII staining of the lymphangioma. The endothelial cells are strongly stained.

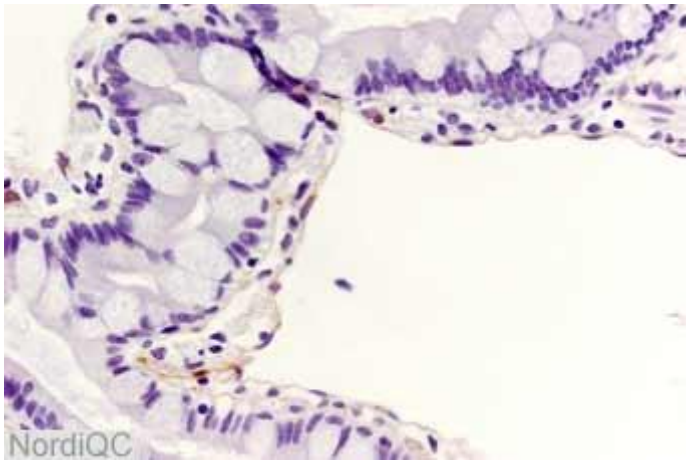


Fig. 3b
Insufficient FVIII staining of the lymphangioma. The endothelial cells are almost negative.

SN/MV/LE 18-6-2004