Assessment Run 32 2011
CD31

Material

The slide to be stained for CD31 comprised:

1. Appendix, 2. Tonsil, 3. Liver, 4. Angiosarcoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD31 staining as optimal included:

- A strong and distinct predominantly membranous staining of the normal vascular endothelial cells and the plasma cells in all the specimens.
- An at least weak to moderate, distinct membranous staining of the activated B- and T-cells – in particular the mantle zone B-cells in the tonsil and the intraepithelial T-cells in the appendix.
- An at least weak to moderate, distinct staining of the majority of the hepatic sinusoidal endothelial cells.
- An at least moderate predominantly membranous staining of the majority of the angiosarcoma.

167 laboratories participated in this assessment. 60% achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD31, run 32**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **JC70A**	95 11 2 1 1 1 1	Dako NeoMarkers Immunologic Biocare Cell Marque IDlabs Master Diagnostica	33	37	30	12	62 %	70 %
mAb clone **1A10**	8 2 1	Leica/Novocastra Zytomed Vector	-	-	6	5	0 %	0 %
pAb **RB-10333-P**	1	Neomarkers	-	-	-	1	-	-
Ready-To-Use Abs								
mAb clone **JC70A, IR610**	16	Dako	7	8	1	0	93 %	100 %
mAb clone **JC70A, IS610**	2	Dako	0	2	0	0	-	-
mAb clone **JC70A, 760-4378**	11	Ventana	1	7	3	0	73 %	-
mAb clone **JC70A, PM131-97**	1	Cell Marque	0	1	0	0	-	-
mAb clone **JC70A, 760-4378**	3	Cell Marque	0	2	1	0	-	-
mAb clone **JC70A, MS-353-R7**	2	Neomarkers	0	0	2	0	-	-
mAb clone **JC70A, PM131**	1	Biocare	0	1	0	0	-	-
mAb clone **1A10 760-4246**	5	Ventana	0	0	0	5	0 %	0 %
mAb clone **1A10 PA0250**	2	Leica/Novocastra	0	0	0	2	-	-
Total	167		41	58	43	25	-	

Proportion			25 %	35 %	26 %	15 %	60 %	

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

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **JC70A**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (3/16)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/4), TRS pH 9 (Dako) (2/9), TRS pH 6.1 (Dako) (9/15), Bond Epitope Retrieval Solution 2 (BERS 2) (Bond, Leica) (8/15) or Cell Conditioning 1 (CC1) (BenchMark, Ventana) (9/30). The mAb was typically diluted in the range of 1:10– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 50 out of 71 (70 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

Ready-To-Use Abs
mAb clone **JC70A** (prod. no. IR610, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using TRS pH 9 or TRS pH 9 (3-in-1), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000/K8002) as the detection system. Using these protocol settings 15 out of 15 (100 %) laboratories produced a sufficient staining.

mAb clone **JC70A** (prod. no. 760-4378, Ventana): The protocol giving an optimal result was based on HIER using standard CC1, an incubation time of 32 min in the primary Ab and UltraView (760-500) with amplification kit (760-080) as the detection system.

The most frequent causes of insufficient stains were:

- Less successful antibodies
- Too low concentration of the primary antibody
- Insufficient HIER – use of less successful retrieval buffer
- Inappropriate retrieval - proteolytic pre-treatment
- Use of low sensitivity detection systems
- Unexplained (10 out of the 42 protocols assessed as insufficient apparently used appropriate settings)

In this assessment a sufficient staining result could only be obtained with the mAb clone JC70A using HIER. In concordance to the previous NordiQC assessments of CD31 the prevalent feature of the insufficient results was a too weak or false negative staining of the cells expected to be demonstrated, which was the case in 67 out of the 68 insufficient staining results (99%). The majority of the laboratories were able to demonstrate CD31 in high antigen expressing structures such as the endothelial cells of the large vessels in the appendix and in the portal tracts of the liver, whereas the demonstration of CD31 in the hepatic sinusoidal endothelial cells and the activated B-cells in the mantle zones with a low expression of CD31 was more challenging and required an optimally calibrated protocol.
The overall pass rate was highly influenced by the Abs, pre-treatment and the detection system used.
All 18 out of 18 protocols (100%) based on the mAb clone 1A10 either as concentrate or as a Ready-To-Use (RTU) format were assessed as insufficient. In these protocols only the endothelial cells of the large vessels in the appendix and in the portal tracts of the liver could be demonstrated, whereas the hepatic sinusoidal endothelial cells and the activated B-cells in the mantle zones were false negative. The constantly poor staining results of the mAb clone 1A10 were seen despite the use of protocol settings comparable to those used for clone JC70A (such as efficient HIER, high sensitive detection systems etc.) that gave optimal results.

The mAb clone JC70A was the most commonly used Ab and the most successful. A total of 148 laboratories used this mAb either as a concentrate or as RTU, out of which 99 (67 %) obtained a sufficient staining result. All 5 out 5 protocols based on enzymatic pre-treatment were assessed as insufficient and only HIER could be used to obtain a sufficient staining. Focusing on the HIER buffer, the highest proportion of optimal scores was obtained by TRS pH 6.1 (Dako) as 12 out of 15 (80%) were assessed as sufficient, of which 9 were optimal (60%). No participants were able to obtain optimal result using other low pH / citric based buffers, and the overall pass rate for these labs was low, namely 5 out of 11 (45%). In comparison HIER in alkaline buffers as e.g. Tris-EDTA/EGTA pH 9.0 gave a higher overall proportion of sufficient results, namely 52 out of 79 (66%) of which 34 were optimal (43%).
Applying the mAb clone JC70A as a concentrate, the pass rates were highly influenced by the sensitivity of the detections systems used. If a 2-step polymer/multimer (e.g. EnVision Flex/UltraView) based system was used, 20 out of 39 obtained a sufficient staining result (53%) out of which 4 (11%) were optimal. If a more sensitive 3-step polymer/multimer (e.g. EnVision Flex+/UltraView + amplification) based system was used, 19 out of 20

obtained a sufficient staining result (95%) of which 17 (85%) were optimal. This calculation is based on optimal protocol settings such as efficient HIER and use of the mAb JC70A in the dilution range 1:10-1:100. The overall pass rate of the mAb clone JC70A applied as a concentrate was 62%

The most successful and robust assay for CD31 in this assessment was obtained by the Ready-To-Use system based on the mAb clone JC70A / IR610 and IS610 from Dako giving a pass rate of 17/17 (100 %) of the laboratories following the protocol settings recommended by Dako. 7 out of the 17 protocols were optimal (41 %). In comparison the Ready-To-Use system from Ventana, prod. no. 760-4378 based on the same clone gave a pass rate of 73 % (8/11) of which one was optimal (9%). The optimal result was obtained by using amplification kit. The overall pass rate of the mAb clone JC70A as RTU system from Dako and Ventana was 86 %.

The liver showed to be a reliable positive control for CD31, provided that the hepatic sinusoidal endothelial cells revealed a distinct and at least weak to moderate positive membranous staining. The liver cells should be negative. Also tonsil showed to be a reliable control, provided that the activated B- and T-cells – in particular the mantle zone B-cells displayed at least a weak to moderate distinct membranous staining. If these cells were negative or only faintly demonstrated, the neoplastic cells in the angiosarcoma also were negative or only showed an equivocal reaction.

This was the 3rd assessment of CD31 in NordiQC (Table 2). A slight increase in the pass rate has been achieved in this run 32, 2011, compared to run 26, 2009, despite the large number of new laboratories participating for the first time. This may reflect that participants to some extend are following both the general and the tailored recommendations given by NordiQC and supported by the fact that fewer participants (5 out of 167 labs) performed proteolytic pre-treatment in this run compared to Run 11 (13 out of 59) and Run 26 (7 out of 116) – all assessed as insufficient.

Table 2. **Proportion of sufficient results for CD31 in the three NordiQC runs performed**

	Run 11 2004	Run 26 2009	Run 32 2011
Participants, n=	59	116	167
Sufficient results	66 %	52 %	60 %

Conclusion

The mAb clone JC70A is the most robust marker for CD31. In this assessment the RTU formats and systems from Dako and Ventana provided a higher pass rate (86 %) than the in-house protocols based on the concentrate (pass rate 62 %). mAb 1A10 constantly gives poor results.
HIER, preferable in TRS pH 6.1 (Dako) or an alkaline buffer is mandatory to provide an optimal staining reaction for CD31. A high sensitive detection system is beneficial.
Liver is recommended as control: The hepatic sinusoidal endothelial cells must show an as strong as possible staining, while the liver cells must be negative.

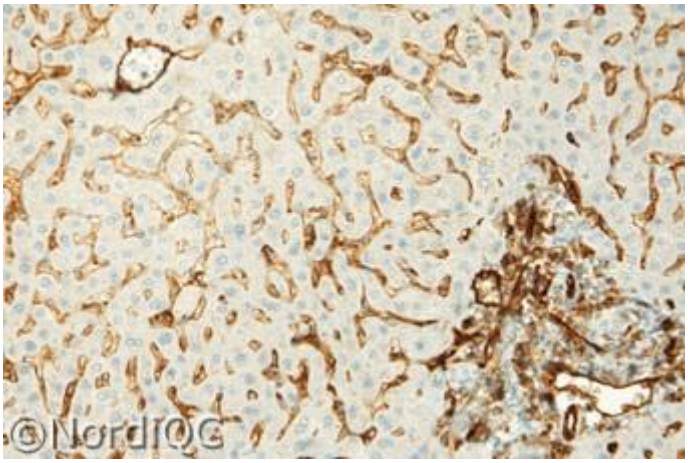

Fig. 1a
Optimal CD31 staining of the liver using the mAb clone JC70A optimally calibrated and with HIER in an alkaline buffer. The hepatic sinusoidal endothelial cells show a moderate predominantly membranous staining reaction, while the endothelial cells of the large vessels in the portal rooms show a strong and intense staining.

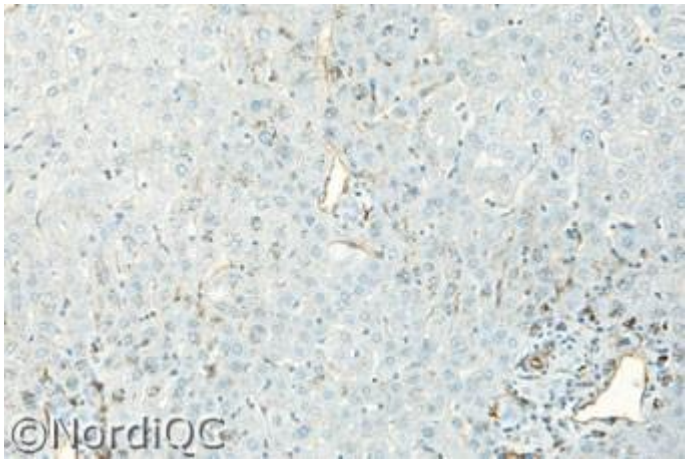

Fig. 1b
Insufficient CD31 staining of the liver using the mAb clone JC70A with proteolytic pre-treatment, same field as in Fig. 1a. Only the endothelial cells of the large vessels show a faint staining, while the endothelial cells of the sinusoids are all false negative. Also compare with Fig. 2b – same protocol.

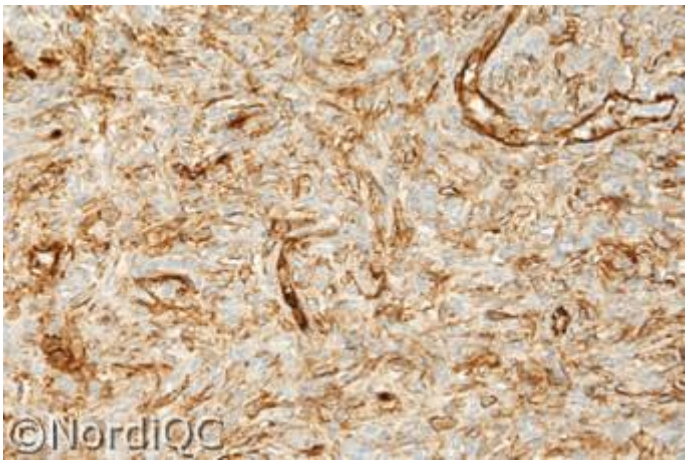

Fig. 2a
Optimal CD31 staining of the angiosarcoma using same protocol as in Fig. 1a. The neoplastic cells show a moderate predominantly membranous staining reaction.

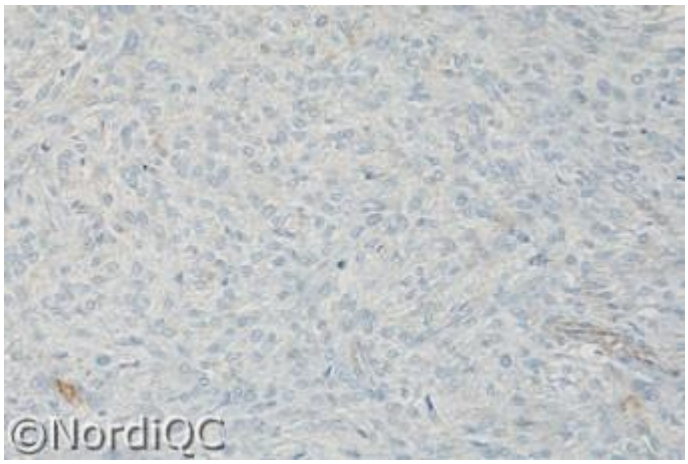

Fig. 2b
Insufficient CD31 staining of the angiosarcoma using same protocol as in Fig. 1b. The neoplastic cells are virtually all false negative, while only the endothelial cells in entrapped normal vessels show a weak staining, same field as in Fig. 2a.

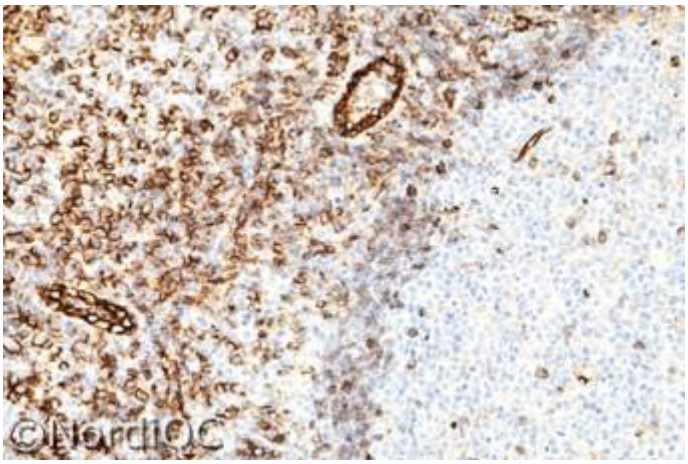

Fig. 3a
Optimal CD31 staining of the tonsil using the mAb clone JC70A optimally calibrated and with HIER in an alkaline buffer/ Bond Epitope Retrieval Solution 2 (Bond, Leica). The B- and T-cells in – in particular the mantle zone B-cells - show a weak to moderate, distinct membranous staining. The endothelial cells show a strong staining.

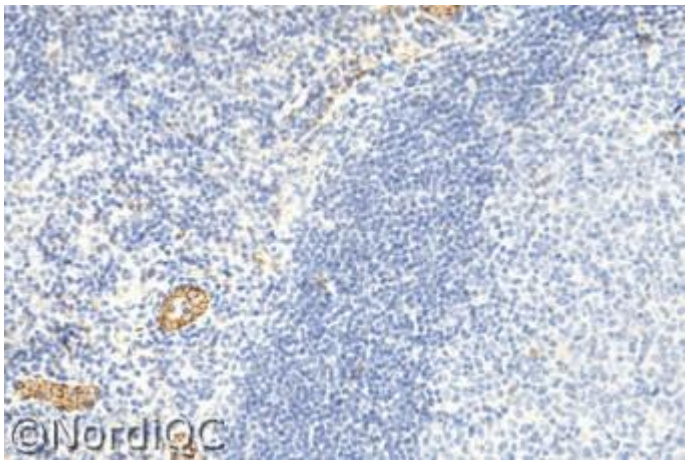

Fig. 3b
Insufficient CD31 staining of the tonsil using same protocol settings as in Fig. 3a except that HIER was performed in a citric based buffer pH 6 / Bond Epitope Retrieval Solution 1 (Bond, Leica). Note, only the endothelial cells of the large vessels show a faint staining, while the B- and T-cells are all false negative, same field as in Fig. 3a.

MB/SN/MV/LE 5-7-2011