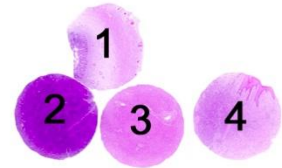
NordiQC

Assessment Run 26 2009
CD31



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 moderate, distinct membranous staining of activated B- and T-cells, particularly the mantle zone B-cells in the tonsil and the intraepithelial T-cells in the appendix.
- An at least moderate, distinct staining of the hepatic sinusoidal endothelial cells.
- An at least moderate, predominantly membranous staining of the majority of the neoplastic cells of the angiosarcoma.
- No staining in the appendiceal epithelial cells and the liver cells.

116 laboratories participated in this assessment. 52 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and scores for CD31, run 26**

Concentrated Abs	**N**	**Vendor**	**Optimal**	**Good**	**Borderl.**	**Poor**	**Suff.**[1]	**Suff. OPS**[2]
mAb clone **JC70A**	83 6 1 1 1 1	Dako NeoMarkers ID Labs Master Diagnostica Sanova SIGNET	16	36	20	21	56 %	77 %
mAb clone **1A10**	3 1	Novocastra NeoMarkers	0	0	1	3	-	-
Ready-To-Use Abs								
mAb clone **JC70A, IR610**	8	Dako	2	5	1	0	88 %	100 %
mAb clone **JC70A, N1596**	1	Dako	0	1	0	0	-	-
mAb clone **1A10, 760-4246**	10	Ventana	0	0	0	10	0 %	0 %
Total	116		18	42	22	34	-	-
Proportion			16 %	36 %	19 %	29 %	52 %	-

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 (5/25)*, Target Retrieval Solution (TRS) pH 6.1 (Dako) (5/15), TRS pH 9 (EnVision FLEX TRS high pH, Dako) (2/8), Bond Epitope Retrieval Solution 2 (Bond, Leica) (3/7) or Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:25– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 37 out of 48 (77 %) 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 (EnVision FLEX TRS high pH, Dako), an incubation time of 20 min in the primary Ab and

EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient stains were:
- Less successful antibodies - e.g., 14/14 protocols based on the mAb clone 1A10 gave an insufficient result
- Inappropriate epitope retrieval - e.g., 7/7 protocols based on enzymatic pre-treatment for the mAb clone JC70A gave an insufficient result
- Too low concentration of the primary Ab.

In this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative reaction of the cells expected to be demonstrated. The majority of the laboratories were able to demonstrate CD31 in the endothelial cells of the large vessels (appendix and hepatic portal tracts). However, the demonstration of CD31 in both the hepatic sinusoidal endothelial cells and the activated B-cells in the mantle zones was only seen with appropriate protocol settings, i.e., a correct titre of the mAb clone JC70A and an efficient HIER. HIER in an alkaline buffer, e.g., Tris-EDTA/EGTA pH 9.1 or TRS high pH 9.0 (Dako) was much more successful than HIER in Citrate pH 6.0, when the clone JC70A was used. Focusing only on the HIER buffer 22/33 (66 %) using one of the 2 alkaline buffers obtained a sufficient result, compared to 6/18 (33 %) when citrate pH 6.0 was used. However the most successful HIER buffer for the clone JC70A was TRS low pH 6.1 (Dako), as 13/15 (87 %) using this HIER buffer with the clone JC70A obtained a sufficient mark - irrespective of the other protocol settings (primary Ab titre, detection system etc.).
In this assessment and in concordance with the results for CD31 in run 11, 2004, it was observed that enzymatic pre-treatment could not be used to give a sufficient staining. In the two runs for CD31 only 1/20 protocols (5 %) using the mAb clone JC70A combined with proteolysis gave a sufficient (good) staining.
When enzymatic pre-treatment is used, this step has to be adjusted to both the fixation time in NBF and the cellular material. Frequently, the lymphocyte membranes were digested to a point where CD31 could not be demonstrated. At the same time, preserved endothelial cells were also negative, indicating that CD31 was not properly retrieved in these cells.
The mAb clone 1A10 was used by 14 laboratories, all of which obtained an insufficient (false negative) result, despite the fact that all the laboratories used protocol settings comparable to the laboratories using the mAb clone JC70A. As 10 of the 14 laboratories used a Ready-To-Use format of the clone 1A10 the less successful performance of the clone can be related to a too low Ab titre in the Ready-To-Use product and does not necessarily mean, that the clone is useless, but this has to be investigated further. 1A10 typically gave a too weak reaction in both the hepatic sinusoidal endothelial cells and the angiosarcoma.

Liver was the most reliable control, in which the hepatic sinusoidal endothelial cells should show an as strong as possible staining, while the liver cells should be negative.
Large vessels (e.g., in the hepatic portal tracts) was less useful as control, as these endothelial cells express a higher concentration of CD31 and will often not reveal suboptimal protocol settings.

This was the second assessment of CD31 in NordiQC. A decrease of the proportion of sufficient results has been seen (table 2). However, there are many new participants for which this was the first CD31 test.

Table 2. **Sufficient results with CD31 in the two NordiQC runs**

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

Conclusion
The mAb clone JC70A is a recommended Ab for CD31. HIER, preferable in an alkaline buffer, or TRS low pH 6.1 (Dako), is mandatory to obtain an optimal result.
Liver is recommended as control: The hepatic sinusoidal endothelial cells must show an as strong as possible staining, without staining of the liver cells.

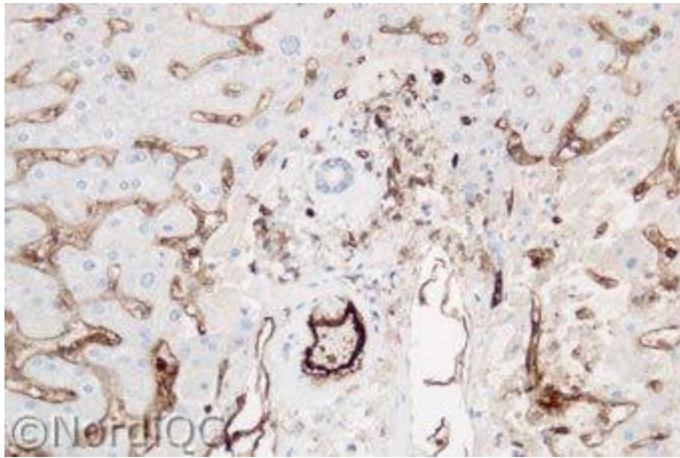


Fig. 1a
Optimal staining for CD31 of the liver using the mAb clone JC70A optimally calibrated and with HIER in TRS low pH, Dako. The endothelial cells lining the vessels in the portal room show a strong staining, while the endothelial cells of the sinusoids show a weak to moderate staining. No background reaction is seen. Also compare with Figs. 2a & 3a – same protocol.

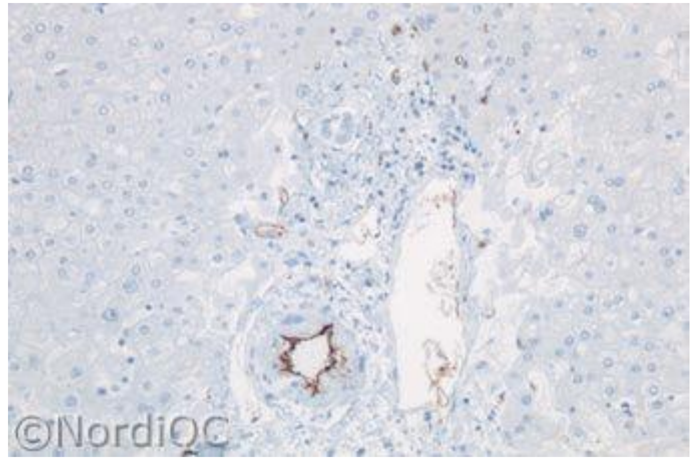


Fig. 1b
Insufficient staining for CD31 of the liver using the mAb clone 1A10 as Ready-To-Use and with HIER in CC1, Ventana. Only endothelial cells of the large vessels show a distinct staining, while the endothelial cells of the sinusoids are all false negative – same field as in Fig. 1a. Also compare with Figs. 2b and 3b – same protocol.

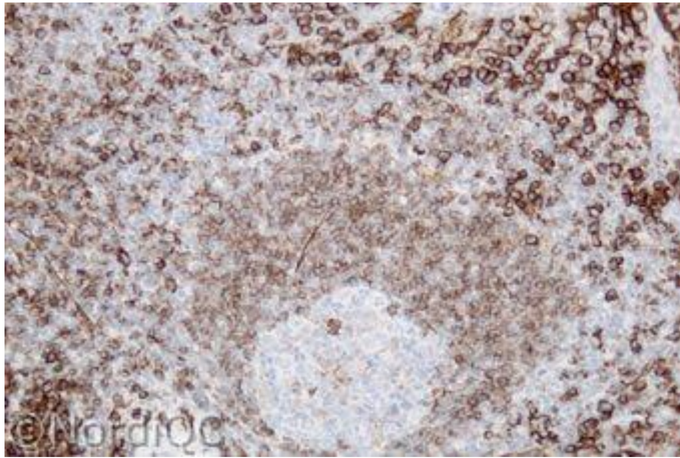


Fig. 2a
Optimal staining for CD31 of the tonsil using same protocol as in Fig. 1a. The activated mantle zone B-cells show a moderate and distinct membranous staining, while the plasma cells (top right) show a strong membranous staining.

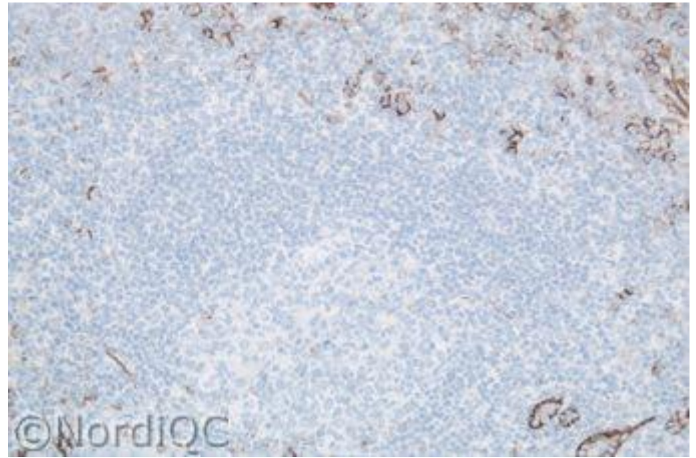


Fig. 2b
Insufficient staining for CD31 of the tonsil using same protocol as in Fig. 1b. Only scattered endothelial cells in large vessels are demonstrated, while the mantle zone B-cells are negative and only few plasma cells are stained - same field as in Fig. 2a. Also compare with Fig. 3b – same protocol.

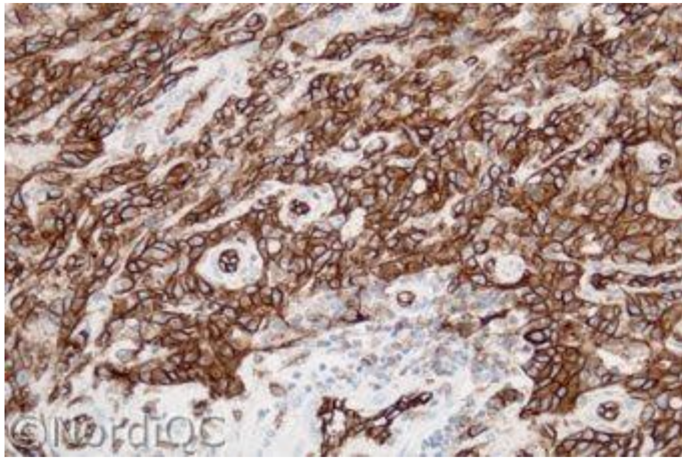


Fig. 3a. Optimal staining for CD31 of the angiosarcoma using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong and distinct predominantly membranous reaction. No background reaction is seen.

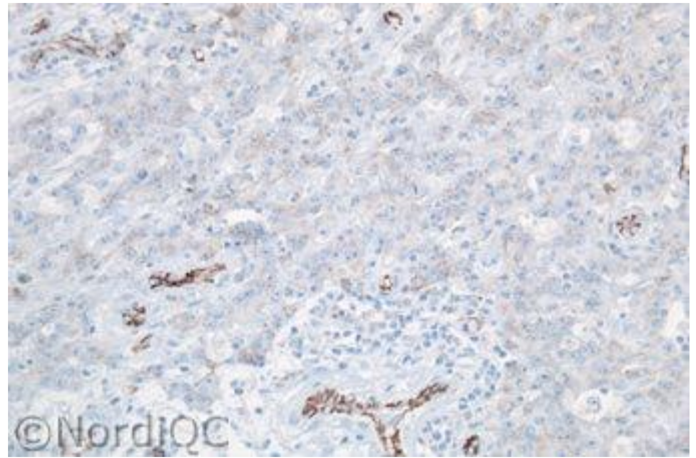


Fig. 3b. Insufficient staining for CD31 of the angiosarcoma using same protocol as in Figs. 1b & 2b. None or only a dubious reaction is seen in the neoplastic cells, while the normal endothelial cells in entrapped vessels show a weak to moderate staining – same field as in Fig. 3a.

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