Assessment Run 31 2011
CD163

The slide to be stained for CD163 comprised:

1. Appendix, 2. Liver, 3. Tonsil, 4. Brain, 5. Spleen histiocytic sarcoma.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD163 staining as optimal included:

- A moderate to strong and distinct cytoplasmic staining of the germinal centre macrophages in the dark zones of the secondary follicles in the tonsil and appendix.
- A moderate to strong cytoplasmic staining of the macrophages in the interfollicular zones of the tonsil, in lamina propria of the appendix and in the Kupffer cells of the liver.
- An at least moderate cytoplasmic staining in virtually all the neoplastic cells of the histiocytic sarcoma.
- No staining in the liver cells and in the epithelial cells of the appendix and tonsil.
- A distinct cytoplasmic staining of the macrophages surrounding the vessels in the brain specimen (the microglial cells express virtually no CD163).

10 laboratories participated in this assessment. 90 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD163, run 31**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **10D**	3 1	Leica/Novocastra Biocare	1	3	0	0	-	-
mAb clone **EDHu-1**	1	Serotec	1	0	0	0	-	-
mAb clone **MRQ-26**	2	Cell Marque	1	1	0	0	-	-
rmAb clone **K20-T**	1	DB Biotech	0	0	1	0	-	-
Ready-To-Use Abs								
rmAb clone **MRQ-26, 760-4437**	2	Ventana	1	1	0	0	-	-
Total	10		4	5	1	0	-	-
Proportion			40 %	50 %	20 %	-	90 %	-

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 **10D6**: The protocol giving an optimal result was based on heat induced epitope retrieval (HIER) using Target Retrival Solution pH 9 (TRS; Dako) (1/2)* as the retrieval buffer. The mAb was diluted 1:200 and EnVision Flex, Dako was used as detection kit.
*(number of optimal results/number of laboratories using this buffer)

mAb clone **EDHu-1**: The protocol giving an optimal result was based on HIER using TRS pH 9 (Dako) (1/1) as the retrieval buffer. The mAb was diluted 1:200 and EnVision Flex, Dako was used as detection kit.

mAb clone **MRQ-26**: The protocol giving an optimal result was based on HIER using standard Cell Conditioning 1 (CC1;Ventana) (1/1) as the retrieval buffer. The rmAb was diluted 1:40 and UltraView + amplification was used as detection kit.

Ready-To-Use Abs
rmAb clone **MRQ-26** (prod. no. 760-4437, 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 + amplification kit as the detection system.

The insufficient staining was charaterized by a weak or equivocal staining of both the normal macrophages and in the neoplastic cells of the histiocytic sarcoma. It was not possible to evaluate if the insufficient result was related to the protocol settings applied or to the rmAb clone K20-T.

In the assessment for CD68 and CD163 the same multitissue block was used. CD163 seemed to be slightly more specific for macrophages (as no cytoplasmic staining in the epithelial cells was seen, which particularly is seen with the CD68 mAb clone KP1). In the germinal centres CD163 expression was primary seen in the macrophages in the dark zone, whereas CD68 was seen in the macrophages in both the dark and the light zone.

Tonsil seems to be a recommendable control for CD163: The interfollicular and germinal centre macrophages in the dark zone must show a moderate to strong cytoplasmic staining. No staining should be seen in the lymphocytes and the squamous epithelial cells.

Conclusion

The mAb clones 10D6, EDHu-1 and the rmAb clone MRQ-26 are all recommendable antibodies for CD163. HIER in an alkaline buffer is mandatory to obtain an optimal result. Tonsil is recommended as control: The interfollicular and dark zone germinal centre macrophages must show a moderate to strong cytoplasmic staining, while the lymphocytes and epithelial cells should be negative.

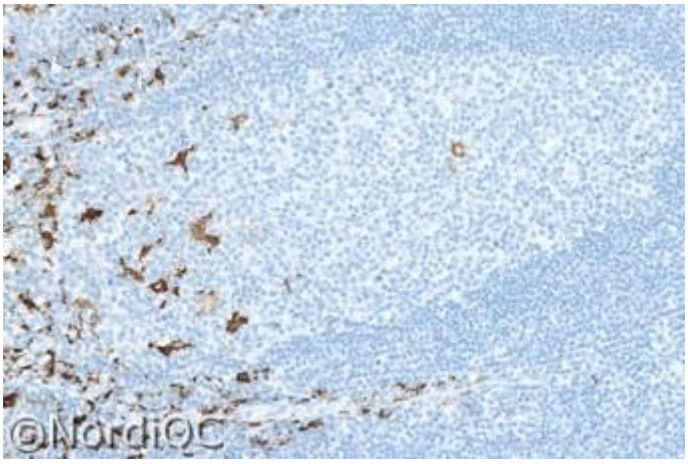

Fig. 1a
Optimal CD163 staining of the tonsil using the rmAb clone MRQ-26 optimally calibrated and with HIER in an alkaline buffer. The germinal centre macrophages in the dark zone and the interfollicular macrophages show a strong cytoplasmic staining.

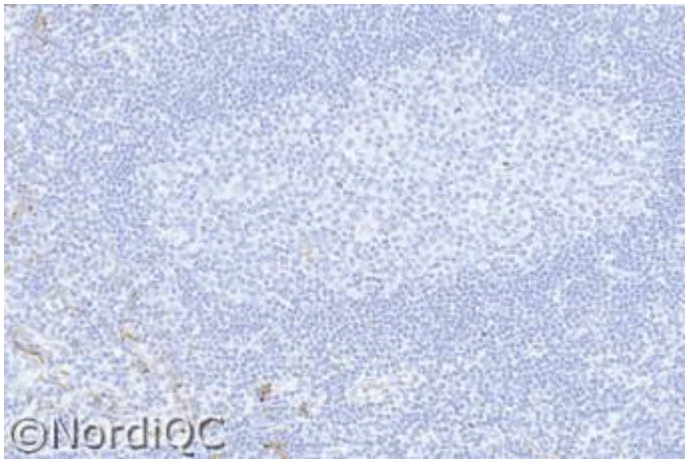

Fig. 1b
Insufficient CD163 staining of the tonsil, same area as in Fig. 1a. Only the interfollicular macrophages show a weak and equivocal staining – also compare with Figs. 2b & 3b same protocol.

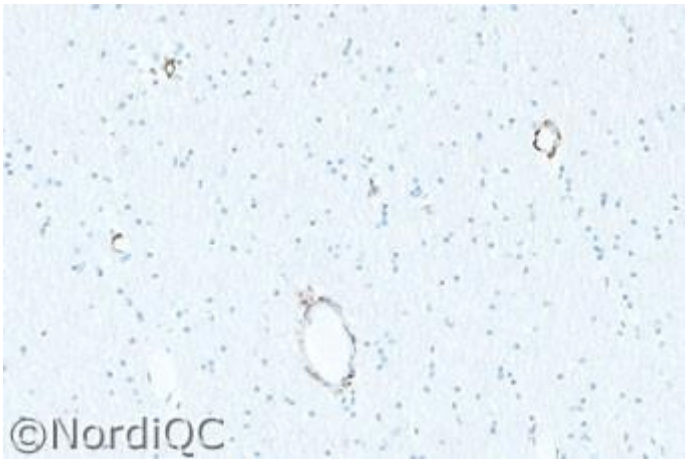

Fig. 2a
Optimal CD163 staining of the brain using same protocol as in Fig. 1a. The perivascular macrophages show a moderate staining reaction, while no staining is seen in the microglial cells.

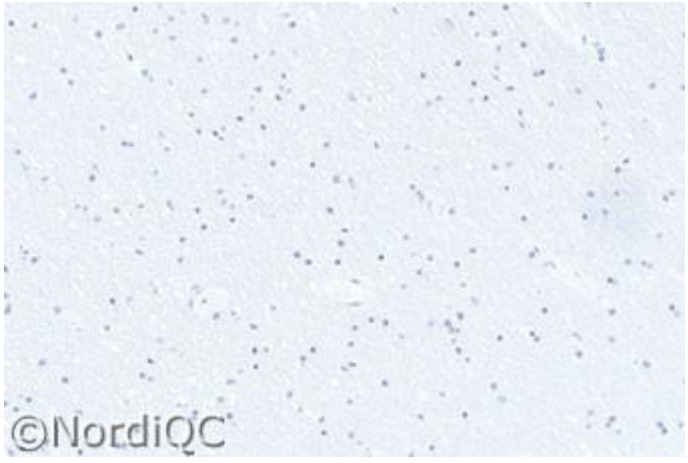

Fig. 2b
Insufficient CD163 staining of the brain using same protocol as in Fig. 1b. No staining is seen in the perivascular macrophages.

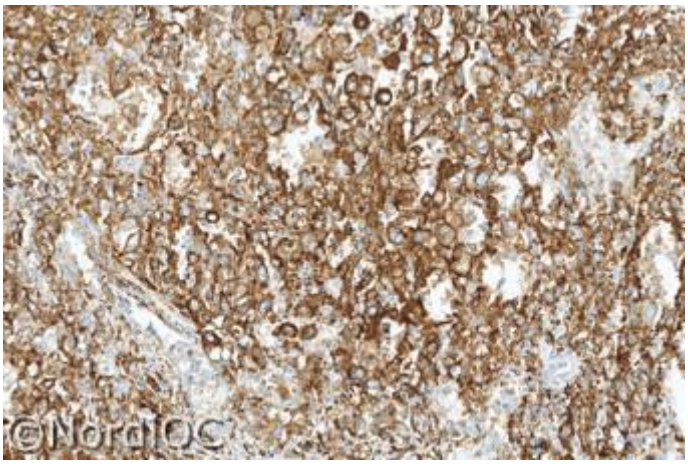

Fig. 3a
Optimal CD163 staining of the histiocytic sarcoma using same protocol as in Figs. 1a & 2a. Virtually all the neoplastic cells show a strong cytoplasmic staining reaction.

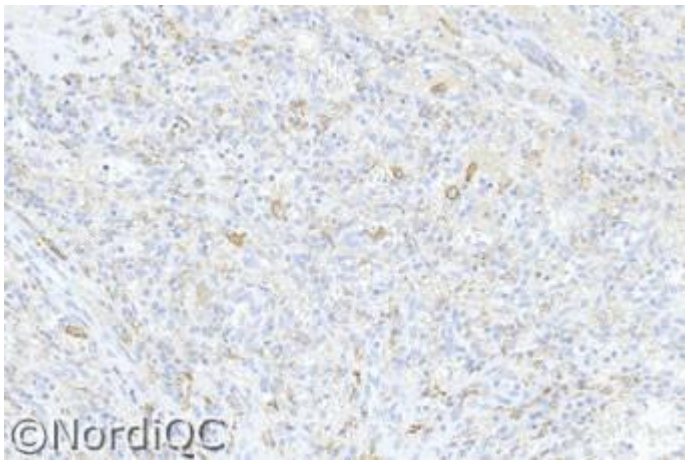

Fig. 3b
CD163 staining of the histiocytic sarcoma using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak cytoplasmic staining reaction.

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