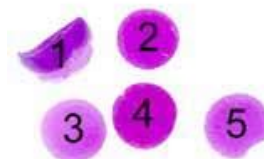


The slide to be stained for CD68 comprised:

1. Appendix, 2. Liver, 3. Juvenile xanthogranuloma, 4. Myeloid leukaemia FAB M4, 5. Meningioma.



Criteria for assessing a CD68 staining as optimal included: A strong and distinct cytoplasmic granular staining of macrophages in the appendiceal germinal centres, hepatic Kupffer cells and macrophages in, e.g., the meningioma, the juvenile xanthogranuloma and the myeloid leukaemia. The neoplastic cells of the meningioma should be negative. A weak reactivity in epithelial cells was accepted with the clone KP1.

64 laboratories submitted stainings. At the assessment 25 achieved optimal staining (39 %), 22 good (34 %), 7 borderline (11 %) and 10 (16 %) poor staining.

The following Abs were used:

- mAb clone KP1 (DakoCytomation, n=36; NeoMarkers, n=1; Ventana, n=1; Zymed, n=1)
- mAb clone PG-M1 (DakoCytomation, n=23)
- mAb clone 54H12 (Novocastra, n=1)

In this assessment optimal stainings could be obtained with the mAb clones KP1 and PG-M1.

11 out of 39 laboratories (28 %) using the clone KP1 and 14 out of 23 (61 %) using the clone PG-M1 achieved an optimal staining.

In the optimal protocols all used HIER, the majority with Tris-EDTA/EGTA pH 9 as the heating buffer (22 out of 46 were optimal), but also Citrate pH 6 (2 out of 9 were optimal) and Target Retrieval Solution (1 out of 3) (DakoCytomation) could be used.

None of the 6 laboratories using proteolytic pretreatment (KP1: n=4; PG-M1: n=2) obtained an optimal staining. KP1 was used in the range of 1:2.000 – 15.000 using Tris-EDTA/EGTA pH 9 (depending on the total sensitivity of the protocols used) and 1:200 using Citrate pH 6 as the heating buffer. PG-M1 was used in the range of 1:100-1:500 with both heating buffers.

The majority of laboratories were able to detect CD68 in the myeloid leukaemia, whereas CD68 in the juvenile xanthogranuloma were only demonstrated in sensitive protocols. The staining patterns for KP1 and PG-M1 were almost identical. However, PG-M1 typically provided a more distinct staining reaction and a better signal-to-noise ratio. The multi-tissue block only comprised a myeloid leukaemia M4. Thus, any difference as regards the reactivity with KP1 and PG-M1 in other subtypes of myeloid leukaemias could not be evaluated.

Using KP1 the appendiceal enterocytes are faintly stained. However, in many insufficient protocols a too high concentration of KP1 was used giving a strong unspecific background reaction in enterocytes as well as various other cell types.

The most frequent causes of insufficient stainings were:

- Inappropriate choice of epitope retrieval (i.e., proteolytic pre-treatment)
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody (KP1).

Controls: Liver and appendix are appropriate controls. In the liver the hepatic Kupffer cells must show an as strong as possible positive staining while the liver cells must be negative. In the appendix the germinal centre histiocytes must show an as strong as possible positive staining while the enterocytes must be negative or show not more than a weak staining (KP1).

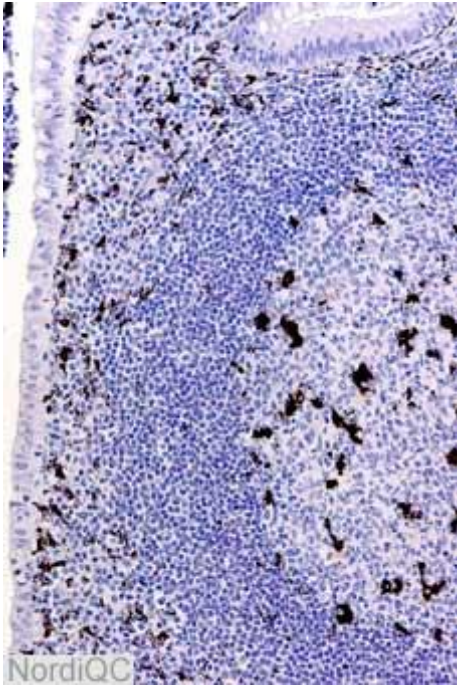


Fig. 1a
Optimal CD68 (clone PG-M1) staining of the appendix. Intense staining is seen in the macrophages, while the enterocytes are unstained.

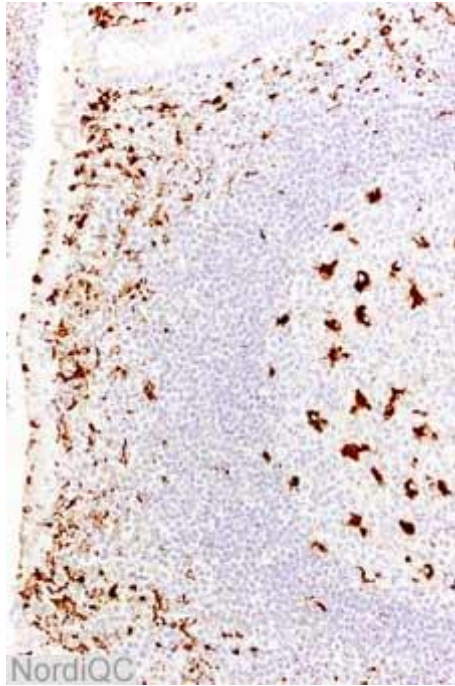


Fig. 1b
Optimal CD68 (clone KP1) staining of the appendix. Intense staining is seen in the macrophages while the enterocytes are weakly stained.

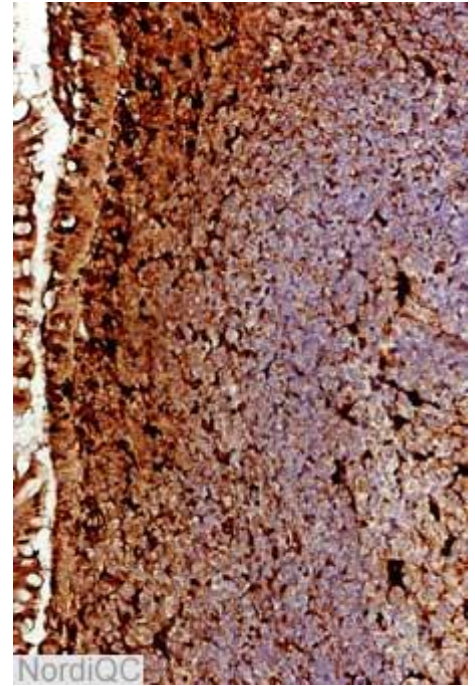


Fig. 1c
Insufficient CD68 (clone KP1) staining of the appendix. Intense staining is seen in the macrophages and enterocytes, but the background reaction is too strong (same field as Fig. 1b).

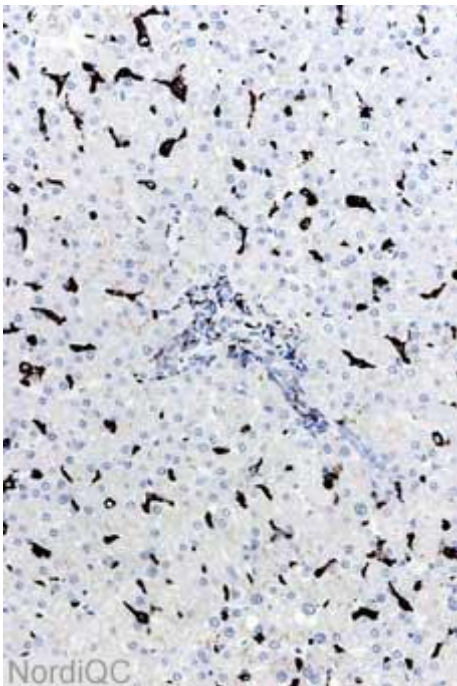


Fig. 2
Optimal CD68 staining of the liver. The Kupffer cells are strongly stained while all other cell types are unstained.

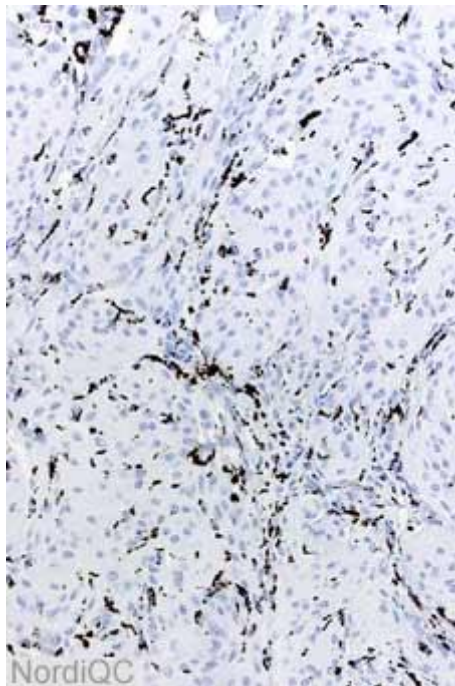


Fig. 3a
Optimal CD68 (clone KP1) staining of the meningioma. Intense staining is seen in macrophages, while the neoplastic cells are unstained.

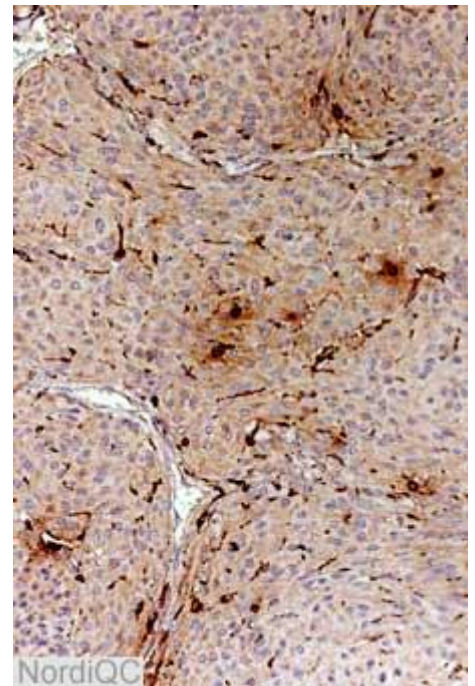


Fig. 3b
Insufficient CD68 (clone KP1) staining of the meningioma. Intense staining is seen in the macrophages, but the neoplastic cells are falsely positive (same protocol as Fig. 1c).

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