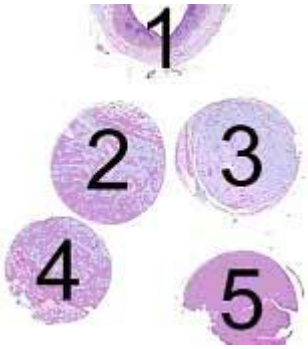
Assessment Run 7 2003
CD34

The slides to be stained for CD34 comprised:
1: appendix, 2-3: gastrointestinal stromal tumours, 4: breast 'myeloid sarcoma' (acute myeloid leukaemia), 5: liver.



Criteria for assessing a CD34 staining as optimal were: a strong and distinct cytoplasmic reaction with membrane accentuation in the endothelial cells of small vessels in the appendix and the liver (portal tracts and zone 1 sinusoids), tumour cells of the GISTs, and a high proportion of tumour cells in the myeloid sarcoma, without any staining of epithelial and smooth muscle cells.

64 laboratories submitted stainings. At the assessment 29 laboratories achieved optimal staining (45%), 19 good (30%), 9 borderline (14%), and 7 poor staining (11%).

57 used mAb QBend10 (DakoCytomation (25), Novocastra (14), Immunotech (6), Neomarkers (4), Ventana (2), Monosan (2), BioGenex (2), Cell Marque (1) and Bio-Zac (1)); 6 used mAb My10 (Becton Dickinson), and one used mAb BI-3C5 (Zymed).

Optimal stainings could be achieved with both mAb QBend10 and mAb My10. The dilution appeared to be highly dependent of origin of the Ab.

Mandatory for an optimal result was HIER. Most optimal protocols used Tris-EDTA/EGTA pH 9, but also citrate pH6 was used in combination with a sensitive visualization system. Laboratories using proteolytic pre-treatment or no pre-treatment obtained good (but not optimal) results in some cases. While it was possible in most protocols to obtain a proper staining of endothelial cells in large vessels, staining of small vessel endothelium, one of the GISTs and the acute myeloid leukaemia provided an optimal protocol.

The most frequent causes of insufficient stainings (often in combination) were:
- A too low primary Ab concentration
- An insufficient HIER (too low pH and/or too short heating time)
- A less sensitive visualization system

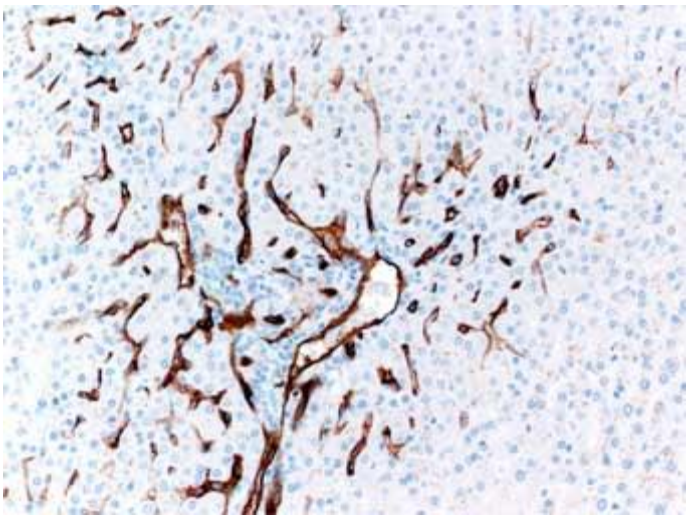


Fig. 1a
Optimal staining for CD34 in a normal liver. Endothelium of portal vessels and periportal sinusoids stains intensely.

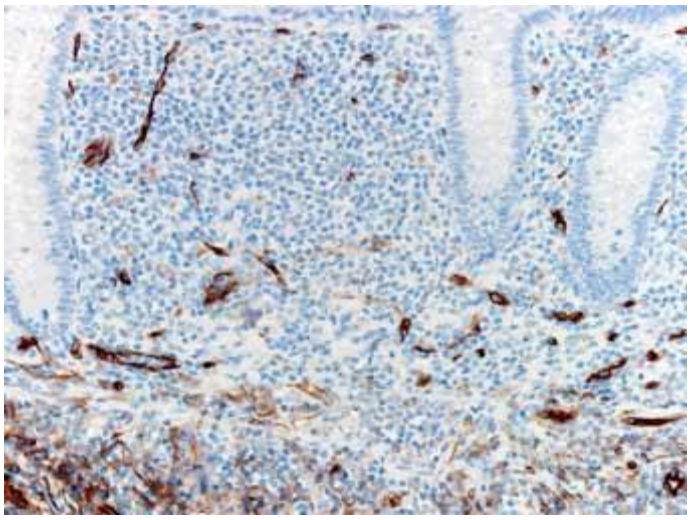


Fig. 1b
Optimal staining for CD34 in a normal appendix. Endothelium of mucosal vessels as well as some stromal cells in submucosa stains intensely.

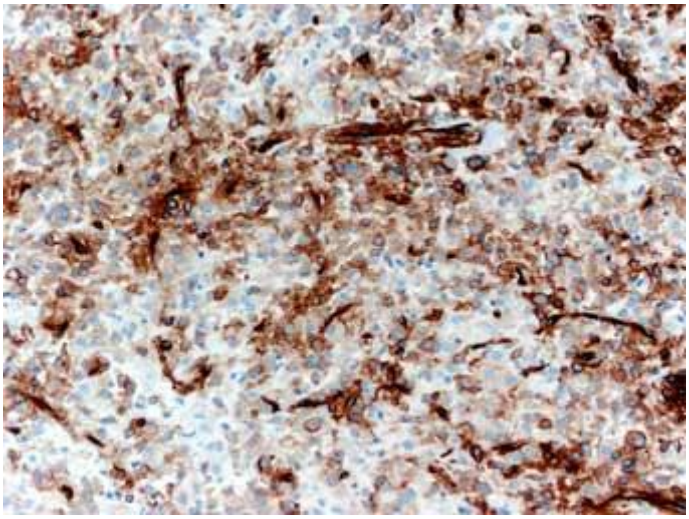


Fig. 1c
Optimal staining for CD34 in a GIST. Endothelial cells stains intensely while the tumour cells in this case give a more heterogenous staining.

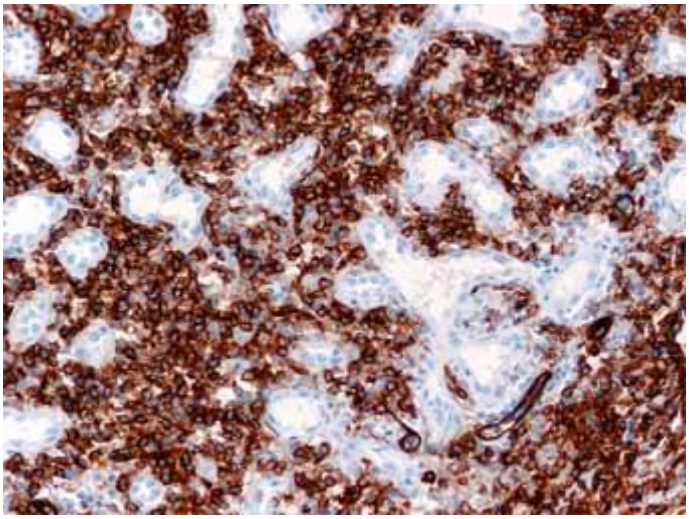


Fig. 1d
Optimal staining for CD34 in a breast infiltrate of acute myeloid leukaemia. Myeloblastic cells stains intensely while the epithelial cells are unstained.

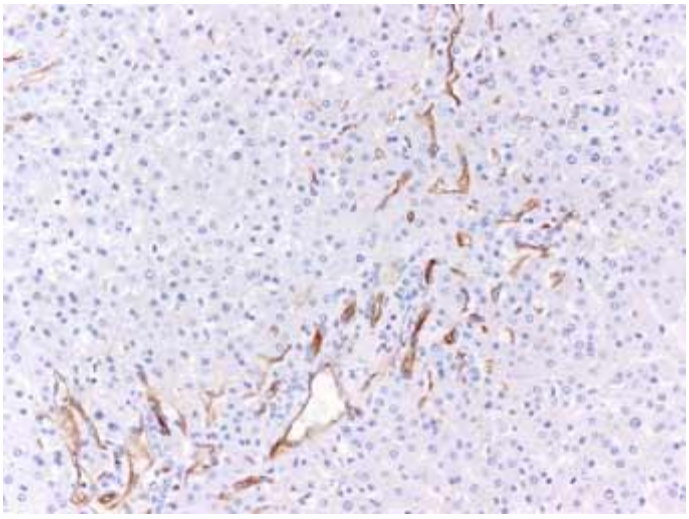


Fig. 2a
Insufficient staining for CD34 in a normal liver, due to insufficient HIER. A moderate staining of endothelium of portal vessels is seen while periportal sinusoids are stained much less than in Fig 1a (same field).

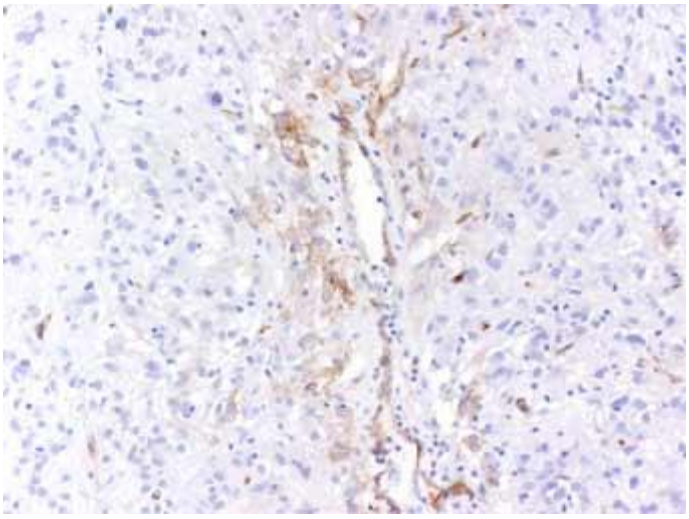


Fig. 2b
Insufficient staining for CD34 in a GIST (same field as in Fig. 1c). Endothelial cells are moderately stained while the tumour cells are weakly stained or unstained.

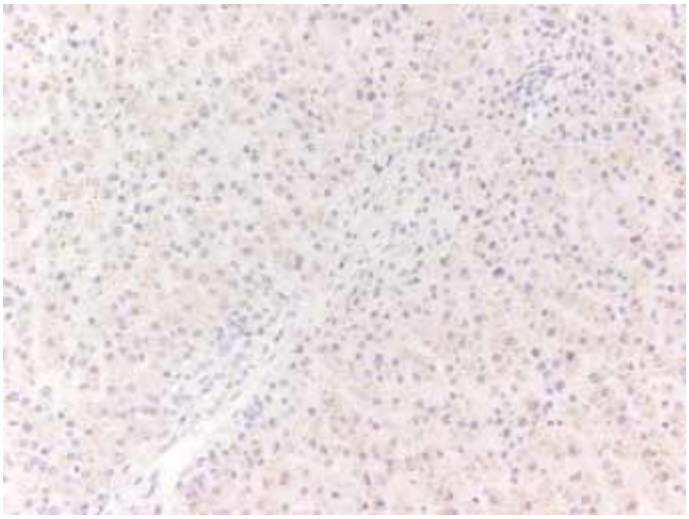


Fig. 3a
Insufficient staining for CD34 in a normal liver (same field as in Fig 1a), due to an inappropriate antibody (mAB BI-3C5). The endothelium is unstained. The staining of liver cells is caused by endogenous biotin.

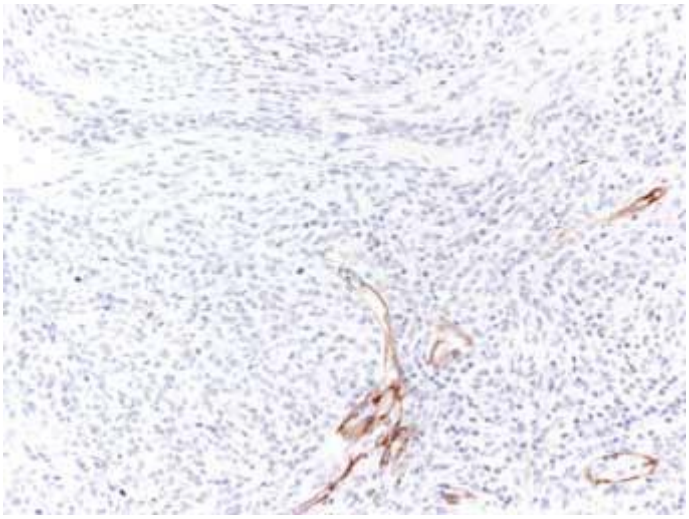


Fig. 3b
Insufficient staining for CD34 in a GIST due to an inappropriate antibody (mAb BI-3C5)(same field as in Fig. 1c). Some staining of endothelial cells is seen while the tumour cells are unstained.

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