The slide to be stained for CD117 comprised:
1: Appendix, 2: Liver, 3: Desmoid, 4-5: Gastrointestinal stromal tumour (GIST).

Criteria for assessing a CD117 staining as optimal included:

- A strong and distinct predominantly membranous but also cytoplasmic staining of mast cells in all specimens and the appendiceal cajal cells as well as the two GISTs
- Generally a negative staining of all other cells and in particular smooth muscle cells. However, some spindle shaped cells in one of the samples of desmoid revealed a staining in some laboratories while not in others, in a pattern that could not be associated to the Abs or protocols. As we have found no explanation for this phenomenon, it has been ignored in the assessment.

87 laboratories submitted stained slides. At the assessment 17 achieved optimal staining (20 %), 56 good (64 %), 10 borderline (12 %) and 4 (5 %) poor staining.

The following Abs were used:
- mAb clone 2E4 (NeoMarkers n=1)
- mAb clone 9.7 (Ventana n=1)
- pAb A4502 (DakoCytomation n=82)
- pAb CMA 767 (Cell Marque n=1)
- pAb RB-1518-P (NeoMarkers n=1)
- pAb SC-168 (Santa Cruz n=1)

Optimal staining in this assessment could only be achieved with the pAb A4502 (17 out of 82 were optimal).

All protocols resulting in an optimal staining were based on HIER with the following buffers: Tris-EDTA/EGTA pH 9 (14 out of 54 were optimal), Citrate pH 6 (1 out of 8 was optimal), TRS pH 9,9 DakoCytomation (1 out of 2 was optimal) and CC1 Ventana (1 out of 9 was optimal). In the optimal protocols the pAb A4502 was used in the range of 1:200 – 1:500 depending on the total sensitivity of the protocol employed.

The combination of the pAb A4502, diluted in the range of 1:200 – 1:500 and HIER in Tris-EDTA/EGTA pH 9 resulted in an optimal staining in 14 out of 33 laboratories (42 %).

The most frequent causes of insufficient staining were (often in combination):
- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Omission of HIER
- Apparently inappropriate choice of primary Ab

The prevalent features of insufficient staining was either a false negative or a false positive reaction in the specimens. The false negative reaction was characterized by either a too weak or totally negative reaction of the appendiceal Cajal cells and the two GISTs. This was typically seen in protocols omitting HIER. The false positive reaction was typically seen in endothelial cells, germinal centre cells and smooth muscle cells, typically appearing with protocols using a too high concentration of the primary Ab. The false positive reaction was present both in protocols with HIER and without HIER.

CD117 was also assessed in run 7, in which 56 laboratories participated. Out of these 38 % (21 laboratories) had an insufficient staining. Each laboratory was given specific recommendations to improve their protocol. 19 laboratories, which obtained an insufficient result in run 7 submitted a new CD117 stain in run 14. 16 out of these followed the recommendation and 15 of them (94 %) improved from insufficient to either good or optimal. 3 laboratories did not follow the recommendations and only one improved.

The overall proportion of insufficient staining was in this run reduced from 38 % in run 7 to 17 % in run 14.
Focusing only on the laboratories participating in both runs (n=51) the proportion of insufficient staining was reduced from 37 % (n=19) to 18 % (n=9).

**Fig. 1a**
Optimal staining of CD117 (pAb A4502) The Cajal cells in the appendiceal muscularis propria are distinctively stained. The smooth muscle cells are unstained.

**Fig. 1b**
Insufficient staining of CD117 (pAb A4502) The Cajal cells in the appendiceal muscularis propria are negative and only mast cells are demonstrated.

**Fig. 2a**
Optimal staining of CD117 in a GIST (pAb A4502). The majority of the neoplastic cells show a strong cytoplasmic reaction with membrane enhancement and focally a dot-like reaction.

**Fig. 2b**
Insufficient staining of CD117 in a GIST (same field as in Fig. 2a). The neoplastic cells are almost negative.
**Fig. 3a**
Insufficient staining of CD117 (pAb A4502) The Cajal cells in the appendiceal muscularis propria are demonstrated, but also muscle cells are positive – probably due to a too high concentration of the primary Ab. The protocol was based on HIER (compare with Fig. 3b).

**Fig. 3b**
Insufficient staining of CD117 (pAb A4502) The Cajal cells in the appendiceal muscularis propria are not distinctively demonstrated, as all structures are stained. This reaction was seen in several protocols omitting HIER in combination with a high concentration of the primary Ab.

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