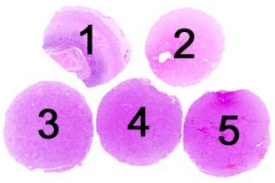
Assessment Run 26 2009
CD117

The slide to be stained for CD117 comprised:
1. Appendix, 2. Desmoid tumour, 3-5. Gastrointestinal stromal tumour (GIST).
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a CD117 staining as optimal included:

- A strong and distinct, predominantly membranous but also cytoplasmic staining of the Cajal cells in the appendiceal muscularis propria.
- A moderate to strong, distinct staining of virtually all the neoplastic cells of the three GISTs.
- A negative reaction of the desmoid tumour.
- A strong predominantly membranous staining of the mast cells in all specimens.
- A negative staining of all other cells (in particular smooth muscle cells). However, a weak reaction in the endothelial cells and peripheral nerves was accepted.

128 laboratories participated in this assessment. 81 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and scores for CD117, run 26**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
pAb **A4502**	113	Dako	29	70	12	2	88%	92%
pAb **RB-9038-P**	2	NeoMarkers	0	0	2	0	-	-
rmAb clone **YR145**	2 1 1	Epitomics Cell Marque Master Diagnostica	3	0	1	0	-	-
Ready-To-Use Abs								
rmAb clone **9.7**	8	Ventana	0	1	7	0	12%	-
mAb clone **T595**	1	Menarini	0	0	0	1	-	-
Total	128		32	71	22	3	-	-
Proportion			25%	56%	17%	2%	81%	92 %

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
pAb **A4502**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Tris-EDTA/EGTA pH 9 (11/32)*, Cell Conditioning 1 (BenchMark, Ventana) (9/24), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako, (6/22) or Bond Epitope Retrieval Solution 2 (Bond, Leica) (3/7) as retrieval buffer. The mAb was typically diluted in the range of 1:100 – 1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 82 out of 89 (92 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

rmAb clone **YR145**: all three protocols giving an optimal result were based on HIER using either Tris-EDTA/EGTA pH 9 (1/1), EDTA/EGTA pH 8 (1/1) or Cell Conditioning 1 (BenchMark, Ventana) (1/1). The rmAb was diluted in the range of 1:200 – 1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Too low or too high concentration of the pAb A4502
- Omission of HIER
- Insufficient HIER (e.g., citrate pH 6.0 or too short heating time).

In this assessment and in concordance with the results in the previous NordiQC assessments of CD117, the prevalent features of an insufficient staining were either a weak/false negative reaction or a false positive reaction in the tested specimens. A weak/false negative reaction was observed in 21 out of 25 (84%) of the insufficient results. These were characterized by a too weak reaction of the appendiceal Cajal cells (which in the previous NordiQC assessments have been identified as the most appropriate positive control for CD117. The false positive reactions (due to a too high Ab concentration) was typically seen in the smooth muscle cells of lamina muscularis propria of the appendix and in the smooth muscle cells of the large vessels. In 3 laboratories the desmoid tumour was distinctively stained, while the other structures stained as expected inclusive a negative reaction of the smooth muscle cells. This aberrant pattern was also noticed in the previous CD117 run 21, 2007 and is most likely related to a lot-to-variation of the pAb A4502 (Dako). Both in the previous and current CD117 run, lot OHO12A has been shown to give this aberrant reaction in the desmoids tumours.
Using pAb A4502 the peripheral nerves in the appendix frequently showed a moderate cytoplasmic reaction, most pronounced when a high concentration (1:20 – 1:300) of the Ab was applied. The reaction pattern was seen with different HIER settings like HIER in Citrate pH 6.0 and Tris-EDTA pH 9.0 and observed with various lots of A4502. The nerves were negative in all protocols based on the rmAb YR145.

This was the fourth assessment of CD117 in NordiQC. A relatively constant proportion of sufficient results have been seen in the latest three runs, as shown in table 2:

Table 2. **Sufficient results with CD117 in four NordiQC runs**

	Run 7 2003	Run 14 2005	Run 21 2007	Run 26 2009
Participants, n=	56	87	118	128
Sufficient results	63%	84%	78%	81%

In the previous assessments of CD117 (run 7, 14 and 21), a total of 61 laboratories obtaining an insufficient result were given specific recommendations how to improve their protocol for CD117. 47 laboratories submitted a new stain in a subsequent run. 35 followed the recommendation, of which 28 improved to good or optimal (80 %). 12 laboratories did not follow the recommendation, and only 3 of these (25 %) obtained a sufficient staining in the subsequent run. 4 laboratories changed their entire system and obtained a sufficient result.

Conclusion
pAb A4502 and rmAb clone YR145 are both recommendable Abs for CD117 in GIST. As regards A4502, however, some lot-to-lot variation has been revealed. HIER, preferable in an alkaline buffer, is mandatory for an optimal result. Concentration of the primary Ab should be carefully calibrated.
Appendix is an appropriate control for CD117: The Cajal cells must show a distinct staining reaction, while the smooth muscle cells in muscularis propria must be negative.

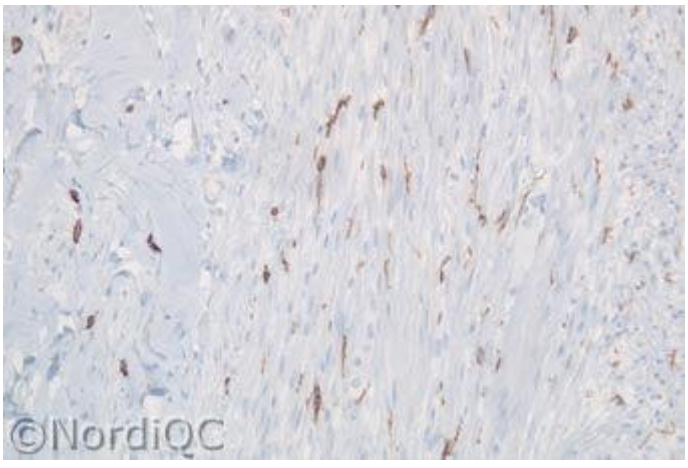


Fig. 1a
Optimal staining for CD117 of the appendix using the rmAb YR145 optimally calibrated and with HIER in an alkaline buffer. The Cajal cells in the appendiceal muscularis propria are distinctively stained. The smooth muscle cells are unstained. Also compare with Figs. 2a & 3a – same protocol.

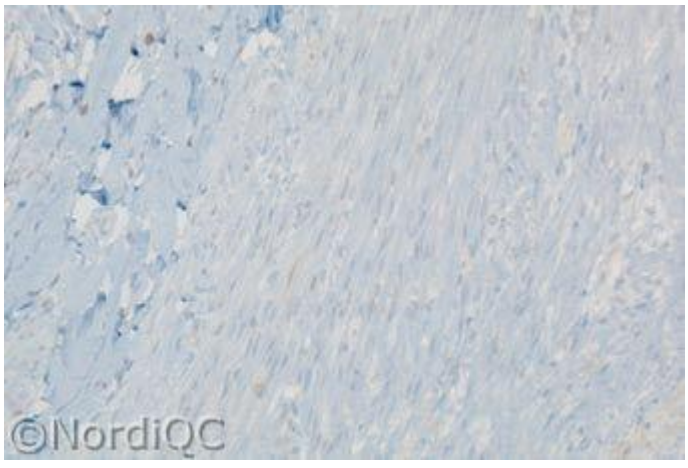


Fig. 1b
Insufficient staining for CD117 of the appendix using the mAb clone T595 as Ready-To-Use and a less successful protocol – same field as in Fig. 1a.
The Cajal cells in the appendiceal muscularis propria are virtually negative. Also compare with Figs. 2b & 3b – same protocol.

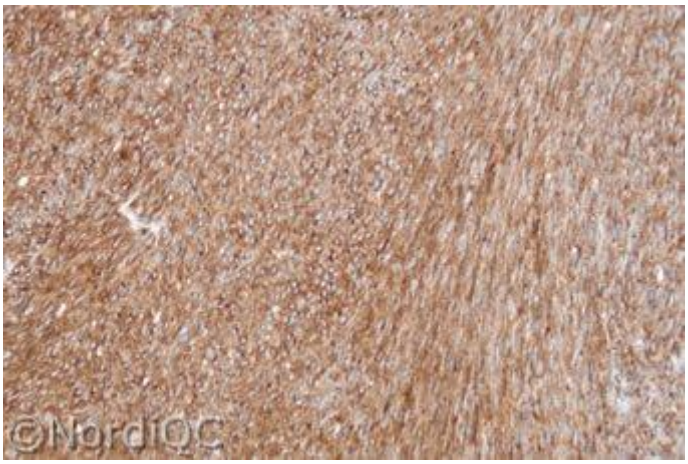


Fig. 2a
Optimal staining for CD117 in a GIST using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong cytoplasmic reaction with membrane enhancement and focally a dot-like reaction.

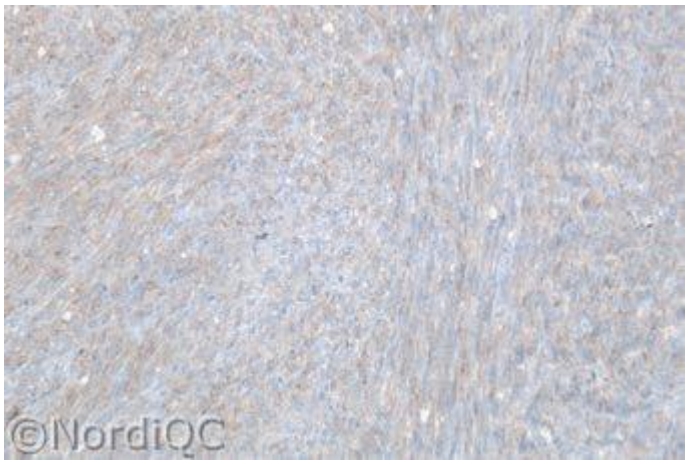


Fig. 2b
Insufficient staining for CD117 in a GIST using same protocol as in Fig 1b. The neoplastic cells only show a weak and diffuse staining – also compare with Fig 2a – same field.

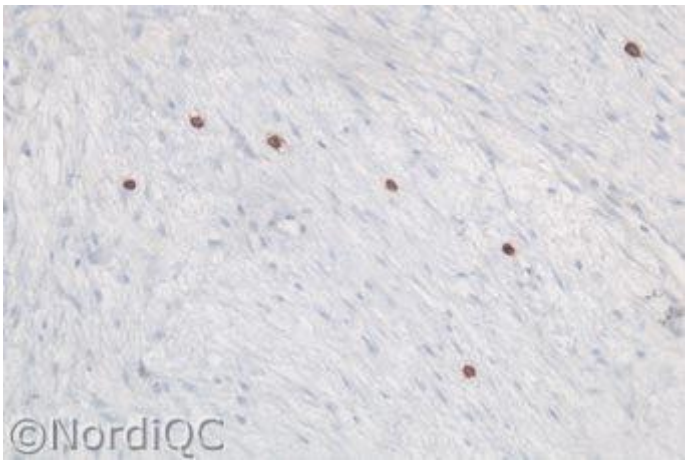


Fig. 3a
Optimal staining for CD117 of the desmoid tumour using same protocol as in Fig. 1a & 2a. Only the mast cells show a distinct predominantly membranous staining, while all other structures are negative.

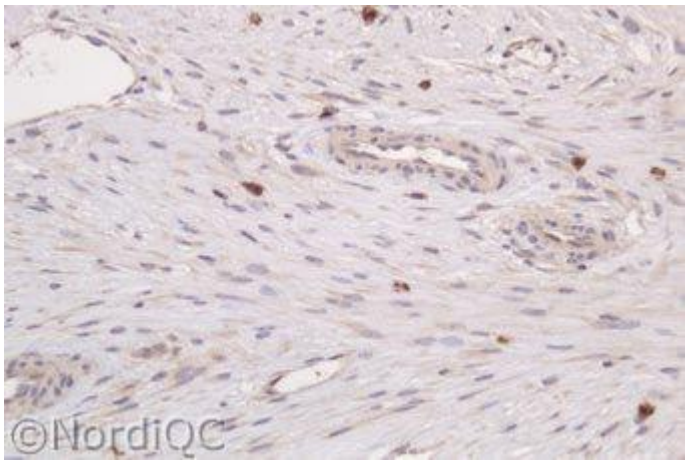


Fig. 3b
Insufficient staining for CD117 of the desmoid tumour using the pAb A4502 too concentrated. Both the smooth muscle cells and the neoplastic cells of the desmoid tumour show a positive staining.

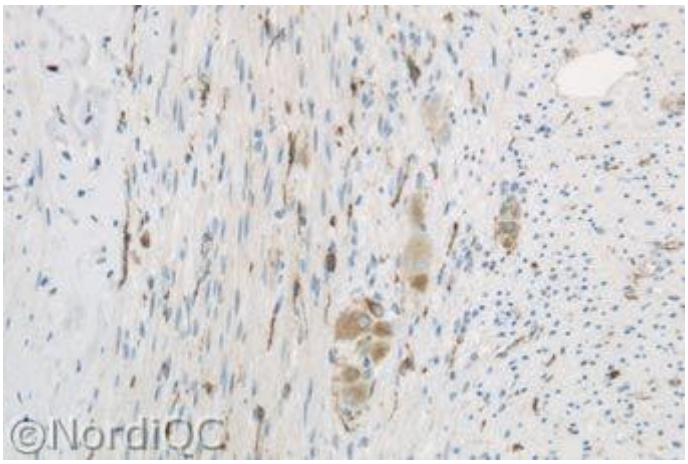


Fig. 4a
Staining for CD117 of the appendix using the pAb A4502. The Cajal cells are demonstrated and the smooth muscle cells are negative. However the peripheral nerves show a distinct positive reaction, which was observed with various lots of the pAb A4502. This staining was based on the lot 10025671.

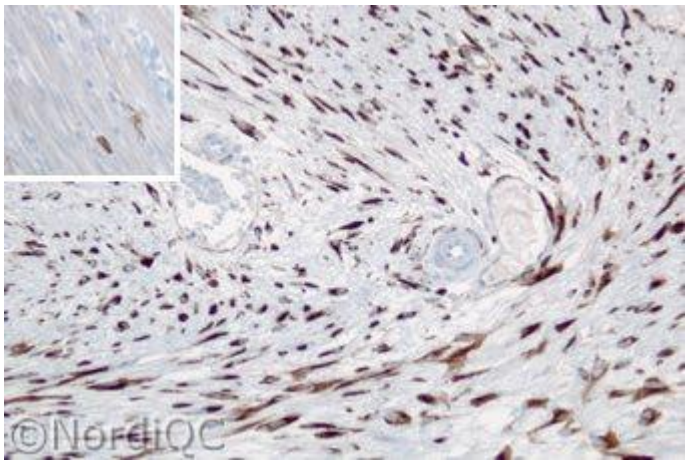


Fig. 4b
Insufficient and aberrant staining for CD117 of the desmoid tumour using the pAb A4502. The neoplastic cells show an intense cytoplasmic staining. This reaction, as noticed and described in the previous assessment, was observed with the pAb A4502 lot no. OHO12A.
The pAb was applied with HIER in an alkaline buffer and a low concentration of the pAb – insert shows the reaction in the appendiceal Cajal cells – stressing the reaction was not caused by use of a too concentrated format of the pAb.

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