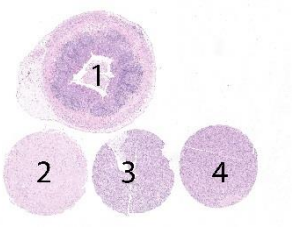
Assessment Run 47 2016
CD117

Material
The slide to be stained for CD117 comprised:
1. Appendix, 2. Desmoid tumour, 3-4. 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 reaction of the Cajal cells in the appendiceal muscularis propria.
- A strong, distinct staining reaction of virtually all neoplastic cells of the GIST tissue core no. 4.
- An at least weak to moderate, distinct staining reaction of the majority of neoplastic cells of the GIST tissue core no. 3.
- A strong predominantly membranous staining reaction of mast cells in all specimens.
- A weak to moderate, distinct staining reaction of neovascular endothelial cells (especially in the desmoid tumour) and epithelial cells in the basal compartment of crypts in the appendix.
- No staining reaction of neoplastic cells of the desmoid tumour and smooth muscle cells of muscularis propria in the appendix.

Participation

Number of laboratories registered for CD117, run 47	287
Number of laboratories returning slides	272 (95%)

Results
272 laboratories participated in this assessment. 129 (47%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low or high concentration of the primary antibody
- Less successful primary antibody - pAb A4502 (Dako) and rmAb 9.7 (Ventana)
- Omission of HIER (protocols based on the pAb A4502)
- Insufficient HIER (e.g., citrate pH 6.0 or too short heating time)
- Less sensitive detection systems (protocols based on the rmAb YR145 and rmAb EP10)
- Unexplained technical issues

Performance history
This was the fifth NordiQC assessment of CD117. The overall pass rate was low and significantly reduced compared to the result obtained in run 26, 2009 (see table 2).

Table 2. **Proportion of sufficient results for CD117 in the five NordiQC runs performed**

	Run 7 2003	Run 14 2005	Run 21 2007	Run 26 2009	Run 47 2016
Participants, n=	56	87	118	128	272
Sufficient results	63%	84%	78%	81%	47%

Conclusion
rmAb clones YR145 and EP10 and pAb A4502 could all be used for optimal demonstration of CD117. The two rmAbs were most successful and provided the highest proportion of optimal results. Efficient HIER, preferably in an alkaline buffer, careful calibration of the primary Ab concentration and use of a 3-step polymer/multimer detection system, is mandatory for an optimal result.
Appendix is an appropriate tissue control for CD117: Cajal cells must show strong and distinct staining reaction, while smooth muscle cells in muscularis propria must be negative. The mast cells must also display a strong staining intensity. A weak to moderate staining reaction of neovascular structures and epithelial cells in the basal compartment of the crypts was typically observed by protocols providing an overall optimal result.

Table 1. **Antibodies and assessment marks for CD117, run 47**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
pAb **A4502**	184	Dako/Agilent	15	73	67	29	48%	59%
pAb **RB-9038-P**	4	Thermo S./Neomarkers	0	1	1	2	-	-
pAb **E1440**	1	Spring Bioscience	0	0	0	1	-	-
rmAb clone **YR145**	10 8 2 2 1 1 1	Nordic Biosite Cell Marque Biocare Medical Immunologic Epitomics Menarini Zeta	14	7	3	1	84%	89%
rmAb clone **EP10**	2	Epitomics	2	0	0	0	-	-
rmAb clone **SP26**	2	Spring Bioscience ZytoMed Systems	0	0	2	0	-	-
mAb clone **T595**	3	Leica/Novocastra	0	0	1	2	-	-
Ready-To-Use antibodies								
rmAb clone **9.7 790-2951**	30	Ventana/Roche	0	4	14	12	13%	0%
rmAb clone **YR145 117R-18**	6	Cell Marque	1	3	1	1	67%	100%
rmAb clone **YR145 RMA-0632**	1	Maixin	1	0	0	0	-	-
rmAb clone **YR145 AN465-5M**	1	Biogenex	0	0	0	1	-	-
rmAb clone **EP10 PA0007**	3	Leica Biosystems	3	0	0	0	-	-
rmAb clone **EP10**	3	Master Diagnostica	2	0	0	1	-	-
rmAb clone **EP10 PME-296**	2	Biocare Medical	0	1	1	0	-	-
pAb **K1907**	2	Dako	0	2	0	0	-	-
pAb **PDR045**	2	Diagnostic Biosystems	0	0	2	0	-	-
mAb clone **T595 PA0900**	1	Leica Biosystems	0	0	0	1	-	-
Total	272		38	91	92	51	-	
Proportion			14%	33%	34%	19%	47%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of CD117, Run 47
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
pAb **A4502**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Cell Conditioning 1 (BenchMark, Ventana) (8/71) *, Target Retrieval Solution (TRS) pH 9 (Dako) (2/30), TRS pH (3-1) (Dako) (1/28) or Bond Epitope Retrieval Solution 2 (Bond, Leica) (4/19) as retrieval buffer. The pAb was typically diluted in the range of 1:100 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 64 of 109 (59%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer).

rmAb clone **YR145**: Protocols with optimal results were all based on HIER using an alkaline buffer as Cell Conditioning 1 (BenchMark, Ventana) (9/13), Target Retrieval Solution (TRS) pH 9 (Dako) (1/2), TRS pH (3-1) (Dako) (1/4), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/2) or Tris-EDTA/EGTA pH 9 (2/2)

as retrieval buffer. The rmAb was typically diluted in the range of 1:25 – 1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 16 of 18 (89%) laboratories produced a sufficient staining.

rmAb clone **EP10**: Two protocols giving optimal results were based on HIER using Cell Conditioning 1 (BenchMark, Ventana) (2/2). The rmAb was diluted in the range of 1:100 – 1:200 depending on the total sensitivity of the protocol employed (UltraView versus OptiView as detection system).

Table 3. **Proportion of optimal results for CD117 for the most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
pAb **A4502**	2/28 ** (7%)	0/6 (0%)	6/58 (10%)	-	3/12 (25%)	0/5 (0%)
rmAb clone **YR145**	0/2	-	6/9 (67%)	-	0/1	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems
rmAb clone **EP10,** product no. **PA0007,** Leica Biosystems BOND III/MAX:
Protocols with optimal results were based on HIER using either BERS1 or BERS2 (efficient heating time 15-20 min.) and 15-20 min. incubation of the primary Ab. Bond Refine (DS9800) was used as detection system. Using these protocol settings all (n=3) protocols provided an optimal result.

rmAb clone **YR145,** product no. **RMA-0632,** Maixin:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time 30 min. at 97°C) and 60 min. incubation of the primary Ab and Maixin, KIT-5230 as detection system.

rmAb clone **EP10,** product no. **MAD-000644-QD,** Master Diagnostica, LAB Vision Autostainer 480:
One protocol with an optimal result was based on HIER using EGTA/EDTA pH 8, 20 min. incubation time of primary Ab and MAD-000237QK (Master Diagnostica) as detection system).

Comments
In this fifth NordiQC assessment of CD117, the prevalent feature of an insufficient staining result was a too weak or false negative staining reaction of cells and structures expected to be demonstrated. This pattern was observed in 83% of the insufficient results (119 of 143). A generally poor signal-to-noise ratio and/or false positive staining reaction in smooth muscle cells of the appendix (muscularis propria and large vessels) and neoplastic cells of the desmoid tumour characterized the remaining insufficient results. The different types of aberrant staining patterns are illustrated in Fig. 6a-d.
Virtually all laboratories were able to demonstrate CD117 in high-level antigen expressing structures such as neoplastic cells of the GIST, tissue core no. 4 and mast cells in all tissue cores included in this assessment. Demonstration of CD117 in low level antigen expressing structures as neoplastic cells of the GIST, tissue core no. 3 and Cajal cells in the appendiceal muscularis propria was significantly more challenging and required a carefully calibrated protocol.

The pAb A4502 was the most widely used antibody for the demonstration of CD117. Used as a concentrate by a laboratory developed (LD) assay, pAb A4502 gave an overall pass rate of 48% (88 of 184). As shown in table 3, optimal results could be obtained on all three main IHC platforms from Dako, Leica and Ventana, although the proportion of optimal scores was low. HIER in an alkaline buffer in combination with careful calibration of the primary Ab were the most critical parameters for a sufficient (good or optimal) result. The overall pass rate for participants using HIER in alkaline buffers e.g. BERS2 (Leica), CC1 (Ventana) or TRS pH 9 (Dako) was 53% (85 of 161) of which 9% (15 of 161) were optimal. In comparison, the overall pass rate for participants using HIER in a citric/acidic based HIER buffer, e.g. Diva Decloaker pH 6.2, TRS pH 6.1 or BERS1, was only 21% (3 of 14) of which none were assessed as optimal. Nine protocols omitted HIER and all (100%) were assessed as insufficient (borderline or poor). There was no significant difference in the overall performance between using a 2-step e.g. Flex (Dako) and UltraView (Ventana)] or 3-step e.g. Flex+ (Dako) and OptiView (Ventana) multimer/polymer based detection system.
As in previous runs for CD117 (run 21, 2007 and run 26, 2009), the pAb A4502 occasionally provided a false positive staining reaction of neoplastic cells in the desmoid tumour. In this run, two protocols based on pAb A4502 gave a distinct staining reaction of the desmoid tumour, while the other structures stained as expected including negative reaction of smooth muscle cells. This aberrant pattern is most likely related

to lot-to-lot-variations of the pAb A4502 (Dako) but no conclusive data could be drawn from this assessment.

In this run, rmAb YR145 provided the best performance within a LD assay for CD117. 84% (21 of 25) of LD assays based on this clone provided a sufficient result of which 56% (14 of 25) were optimal. Efficient HIER in alkaline buffer and most critically use of a sensitive 3-step multimer/polymer detection system was a prerequisite for an optimal result. 86% (18 of 21) of protocols with sufficient score (optimal or good) used HIER in an alkaline buffer e.g. BORG (Biocare Medical), CC1 (Ventana) or TRS pH9 (Dako) and a 3-step multimer/polymer detection system, whereas 100% (4 of 4) of the protocols giving an insufficient score (borderline or poor) used a 2-step mutimer/polymer detection system (e.g. Flex (Dako) and UltraView (Ventana)). Two of the insufficient protocols in addition used less efficient HIER in acidic/citric based buffers.

The newly launched rmAb clone EP10, both as concentrate and Ready-To-Use (RTU) system, also seems promising for demonstration of CD117 (see table 1). Two of two laboratories using the rmAb EP10 within a LD assay produced an optimal result. Both laboratories performed HIER in CC1 (efficient HIER time for 30-40 min.) and used either UltraView with amplification (Ventana) or OptiView (Ventana) as detection system.

Overall the performance of the two rmAbs clone YR145 and EP10 was superior to the pAb A4502 within LD assays. In fact, the proportion of optimal results was significantly higher for rmAbs clones YR145 and EP10 compared to the overall pass rate obtained with the pAb A4502. Grouped together, 59% (16 of 27) of protocols based on rmAb clones YR145 and EP10 provided an optimal result, whereas only 48% (88 of 184) of protocols based on pAb 4502 could produce a sufficient staining result (optimal or good). These data do indicate that it may be advantageous to use these new rabbit monoclonal antibodies to improve the technical and analytical quality for the IHC demonstration of CD117.

In this assessment, the RTU system from Ventana (790-2951) based on the rmAb 9.7, gave a low proportion of sufficient results (see table 1). In the last two runs for CD117 (run 26, 2009 and run 47, 2016), a total of 38 protocols has been assessed and only 13% (5 of 38) produced sufficient results none of which were optimal. Despite using protocol settings producing optimal results with the rmAb YR145 (efficient HIER in CC1 for 32-64 min. and a sensitive detection system as OptiView or UltraView with amplification), the RTU system was less successful and inferior to the rmAb clones YR145 or EP10. Neither vendor recommended nor laboratory modified protocol settings could provide optimal results.

Although the number of protocols submitted for assessment was relative low, the RTU system from Leica, PA0007 based on the rmAb EP10 produced a pass rate of 100% (3 of 3) and all were assessed as optimal. Two optimal results were obtained by using the vendor protocol recommendations based on HIER in BERS2 for 20 min. at 100°C and Bond Refine DS9800 as detection system. One laboratory modified the protocol settings adjusting the HIER conditions using BERS1 and an efficient HIER time for 15 min. at 100°C.

This was the fifth assessment of CD117 in NordiQC (Table 2). A pass rate of 47% was obtained, which is a significant decrease compared to 81% in run 26, 2009. The number of new participants has increased by 113% compared to run 26, 2009 and can in part contribute to the reduced pass rate, but is not the only explanation for the overall poor outcome.
In this run, the performance of the well-established Abs pAb A4502 (Dako) and in particular the rmAb 9.7 (Ventana) was less successful. Overall pass rate of 48% (88 of 184) was seen for pAb 4502 compared to 88% (99 of 113) in the previous run 26, 2009. For rmAb clone 9.7 a pass-rate of 12 and 13% in the two assessments was seen. In contrast, the newly launched clones mAbs YR145 and EP10 provided a significant superior performance. The rmAb clone YR145 was used by NordiQC reference labs as ”reference” to screen tissues for the TMA. Thus, the use of highly sensitive and improved Abs for the establishment of reference data potentially increases the challenge for participants using “old” antibodies and could, in turn, affect the pass rates.

Controls
Appendix is recommended as positive and negative tissue controls for CD117. Cajal cells, mast cells and neovascular structures must be stained as strong as possible without any staining reaction of the smooth muscle cells in lamina muscularis propria or smooth muscle cells surrounding the vessels.

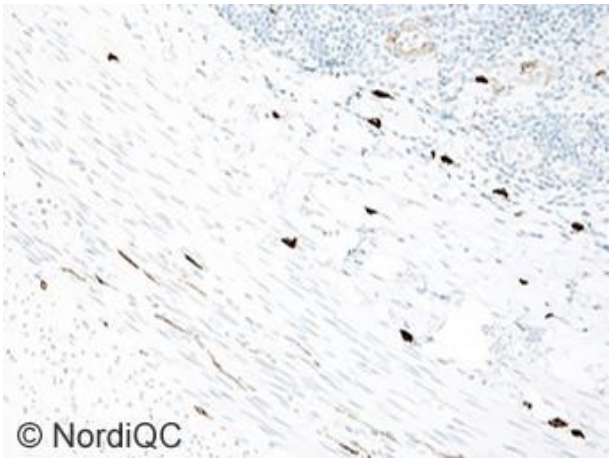


Fig. 1a (x200)
Optimal staining for CD117 of the appendix using the rmAb YR145 as a concentrate, HIER in an alkaline buffer (CC1) and a multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 4a. The Cajal cells in the appendiceal muscularis propria are distinctively stained. The smooth muscle cells are unstained. Also, mast cells display strong intensity and endothelial cells is weakly labelled - compare with Fig. 1b.

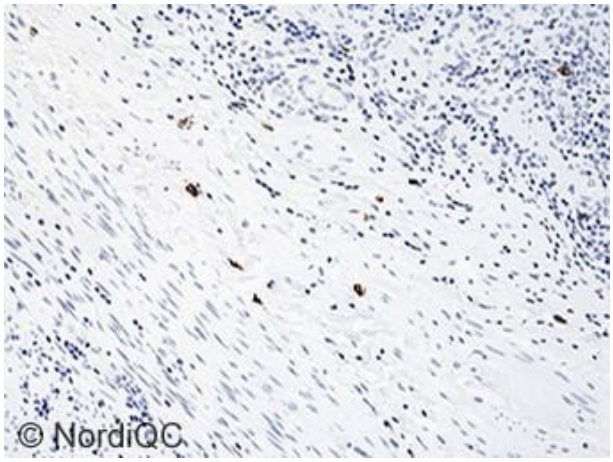


Fig. 1b (x200)
Insufficient staining for CD117 of the appendix using the mAb clone YR145 as concentrate (too diluted), HIER in an alkaline buffer (CC1) and a less sensitive multimer based detection system (Ultraview, Ventana) – same protocol used in Figs. 2b – 4b. The Cajal cells in the appendiceal muscularis propria are completely negative and intensity of both mast cells and endothelial cells is significantly reduced - compare with Fig. 1a (same field).

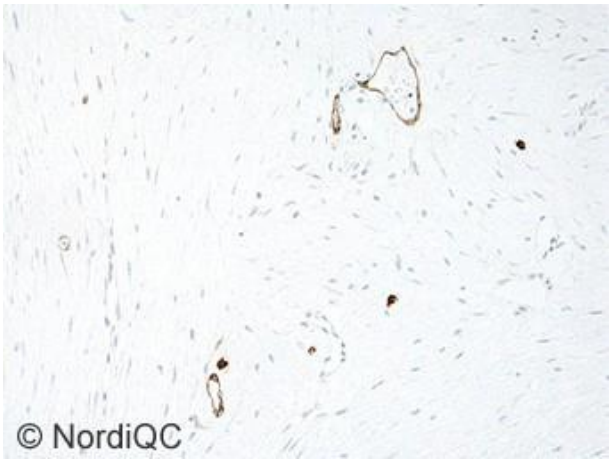


Fig. 2a (x200)
Optimal staining for CD117 in the desmoid tumour using same protocol as in Fig. 1a. Only the mast cells and endothelial cells show a distinct predominantly membranous staining, while all other structures are negative.

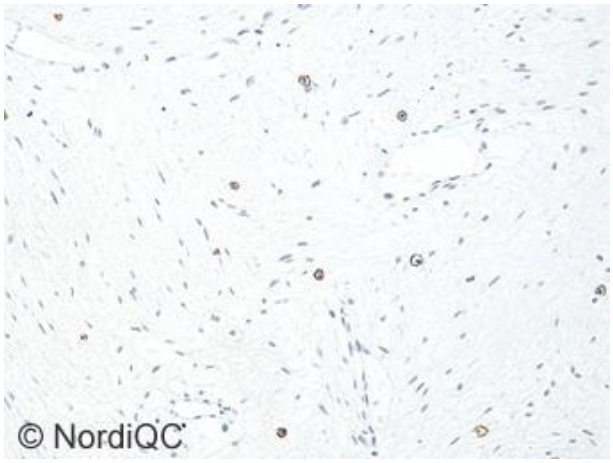


Fig. 2b (x200)
Insufficient staining for CD117 in the desmoid tumour using same protocol as in Fig. 1b. Staining intensity of mast cells is too weak and endothelial cells structures are completely negative - compare with Fig. 2a (same field).

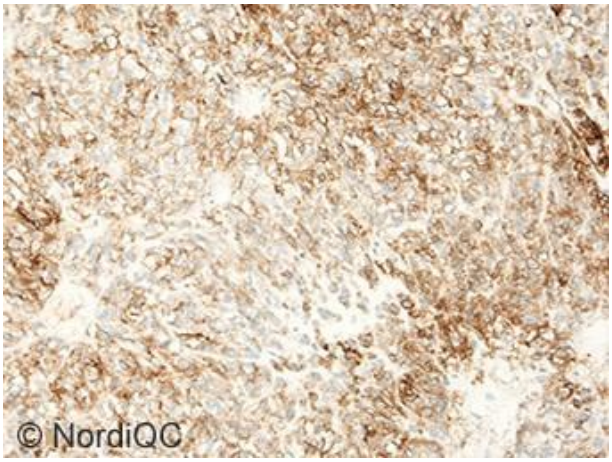


Fig. 3a (x200)
Optimal staining for CD117 of the GIST, core 3 using same protocol as in Fig. 1a & 2a. Virtually all the neoplastic cells show a moderate but distinct staining reaction.

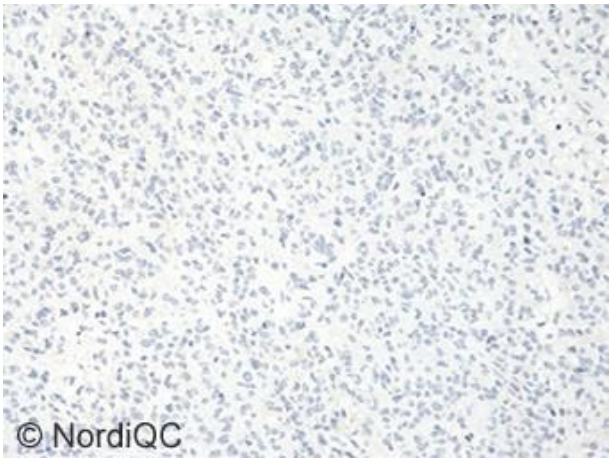


Fig. 3b (x200)
Insufficient staining for CD117 of the GIST, core 3 using same protocol as in Fig. 1b & 2b. The neoplastic cells are completely negative - compare with Fig. 3a (same field).

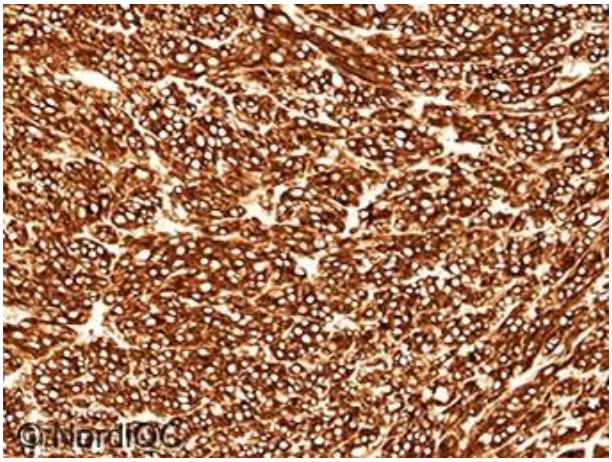


Fig. 4a (x200)
Optimal staining for CD117 of the GIST, core 4 using same protocol as in Fig. 1a - 3a. All the neoplastic cells are strongly stained.

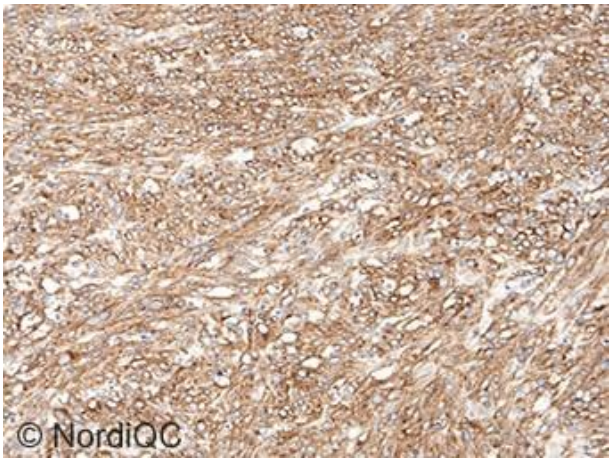


Fig. 4b (x200)
Insufficient staining for CD117 of the GIST, core 4 using same protocol as in Fig. 1b -3b. Staining intensity of the neoplastic cells is significantly reduced - compare with Fig. 4a (same field).

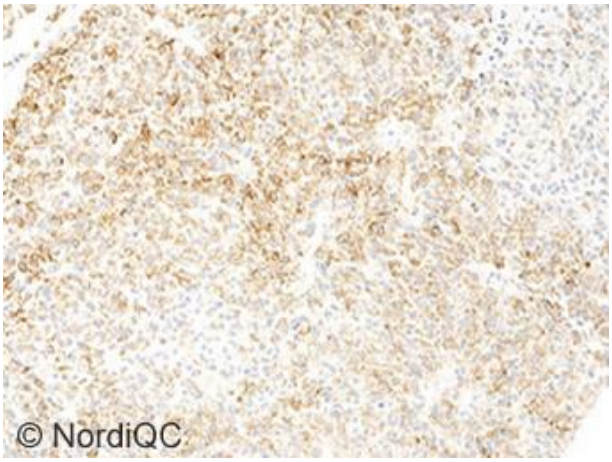


Fig. 5a (x200)
Optimal staining for CD117 of the GIST, core 3 using the rmAb EP10 as Ready-To-Use format (PA0007, Leica Biosystems), with HIER in BERS2 for 20 min, Bond Refine DS9800 as the detection system and performed on the BOND III (Leica Biosystems). The majority of the neoplastic cells show an at least weak to moderate but distinct staining reaction.

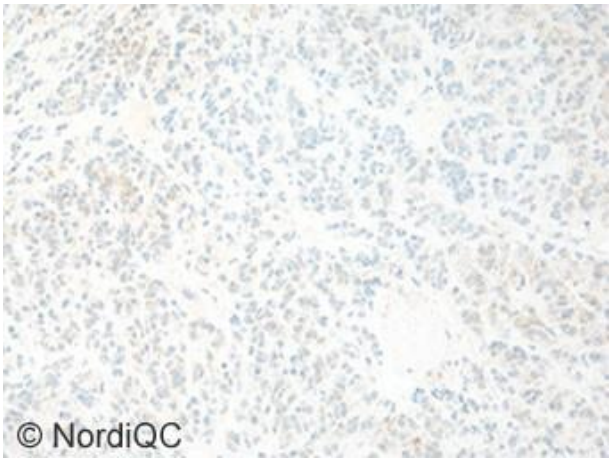


Fig. 5b (x200)
Insufficient staining for CD117 of the GIST, core 3 using the rmAb 9.7 as Ready-To-Use format (790-2951, Ventana), with HIER in CC1 for 64 min, OptiView 760-700 as the detection system and performed on the BenchMark Ultra (Ventana). The neoplastic cells are completely negative or only faintly stained, a typical pattern obtained with this RTU system - compare with Fig. 5a (same field).

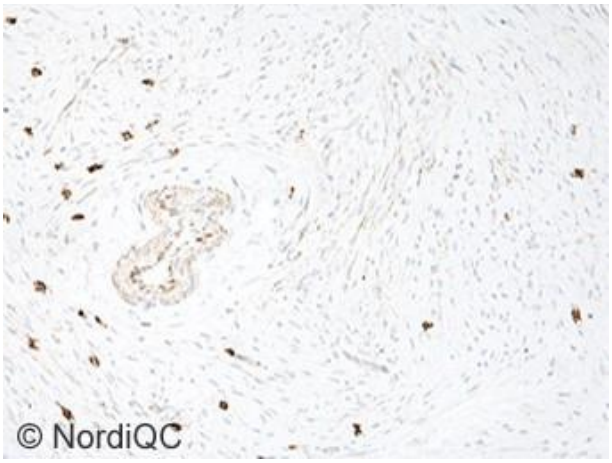


Fig. 6a (x200)
Insufficient staining for CD117 using the rmAb EP10 as Ready-To-Use format (PME-293, Biocare Medical), with HIER in Diva Decloaker for 15 min at 110°C (pressure cooker), a 2-step polymer MACH, MHR534 as the detection system and performed on the IntelliPath (Biocare Medical). The smooth muscle cells surrounding the large vessels and the neoplastic cells of the desmoid tumour are false positive. For the rmAb EP10, this was the only protocol producing this aberrant pattern - compare with Fig. 2a.

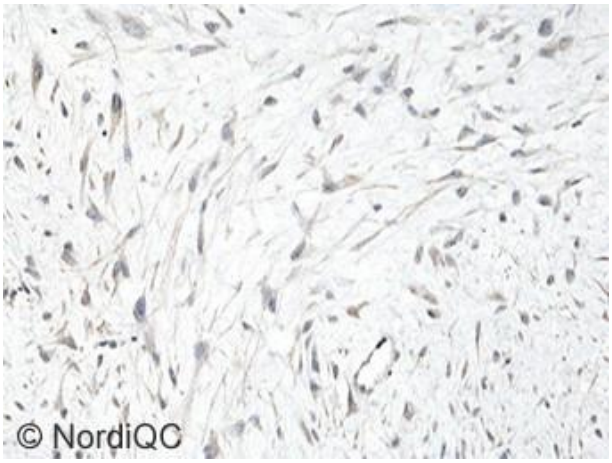


Fig. 6b (x400)
Insufficient and aberrant staining for CD117 of the desmoid tumour using the pAb A4502 (lot no. 10107664, Dako) as concentrate, and with same protocol settings as in Fig. 6a. The neoplastic cells show a distinct cytoplasmic staining reaction - compare with Fig. 2a. This reaction has also been noticed in previous assessments, but primarily related to the pAb A4502 lot no. OHO12A.

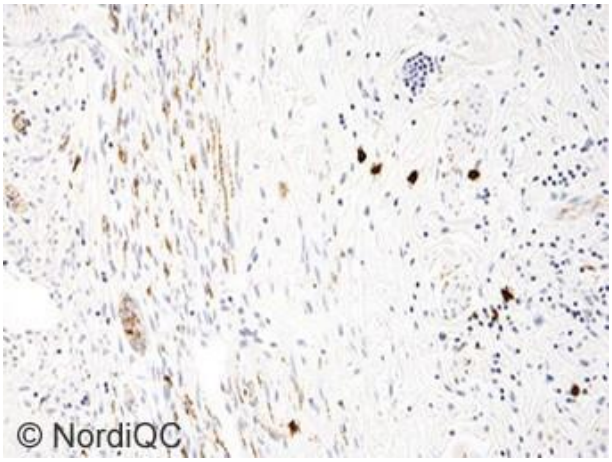


Fig. 6c (x200)
Insufficient and false positive staining for CD117 of the appendix using the pAb A4502 as concentrate, with HIER in BERS2 for 20 min, Bond Refine DS9800 as the detection system and performed on the BOND III (Leica Biosystems). The ganglion cells and the axons of the peripheral nerves are aberrantly stained. The primary antibody is most likely contaminated (vendor or laboratory) with either chromogranin A or synaptophysin - compare with Fig. 1a.

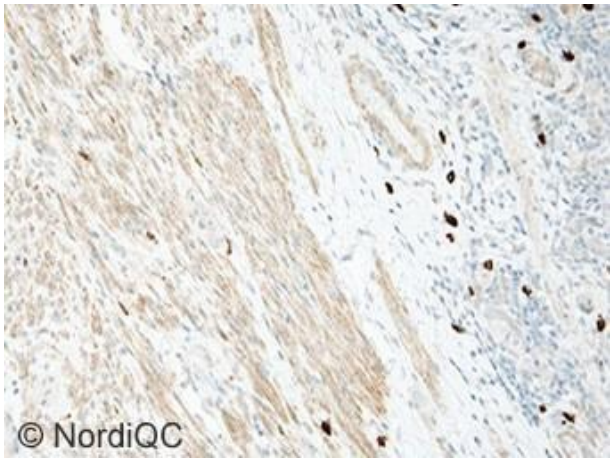


Fig. 6d (X200)
Insufficient and false positive staining for CD117 of the appendix using the pAb A4502 as concentrate. The LD assay is poorly calibrated using a too high concentration of the primary Ab (1:40), efficient HIER in CC1 combined with the use of a high sensitive detection system OptiView 760-700 (Ventana). Smooth muscle cells in the appendiceal lamina muscularis proria and in vessels are aberrantly labelled. The Cajal cells are difficult to identify – compare with Fig. 1a.

MBZ/SN/LE/MV/MV/RR 10.06.2016