Assessment Run 71 2024
CD117

Purpose
Evaluation of the technical performance, and in particular the level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD117, typically used in the diagnostic work-up of gastrointestinal stromal tumors (GISTs) and germ cell tumors. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for CD117 (see below).

Material
The slide to be stained for CD117 comprised:

1. Appendix, 2. Seminoma, 3 and 4. Gastrointestinal stromal tumor (GIST), 5. Schwannoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD117 staining as optimal included:

- An at least moderate, predominantly membranous but also cytoplasmic staining reaction of virtually all Cajal cells in the appendiceal muscularis propria.
- A moderate to strong, distinct membranous staining reaction of all neoplastic cells in the seminoma.
- A moderate to strong, distinct staining reaction of virtually all neoplastic cells in the two GISTs.
- A strong, predominantly membranous staining reaction of mast cells in all specimens.
- No staining reaction in neoplastic cells of the schwannoma.
- No staining reaction of smooth muscle cells (all specimens).

KEY POINTS FOR CD117 IMMUNOASSAYS
- The rmAb clones **EP10** and **YR145** are recommendable Abs.
- The pAb **A4502** performed superiorly compared to the previous NordiQC CD117 assessment runs, however the proportion of optimal results was inferior to rmAbs EP10 and YR145.
- The new Ventana/Roche RTU system **790-7061** based on rmAb clone EP10 performed superiorly compared to the previous Ventana/Roche RTU system based on rmAb clone 9.7.
- RTU systems, especially the Leica Biosystems RTU system **PA0007**, were successful.
- The TMA circulated only consisted of solid tissues with moderate to high expression levels of CD117 and no data was generated on performance of the protocols assessed in other relevant materials as e.g. bone marrow.

Participation

Number of laboratories registered for CD117, run 71	452
Number of laboratories returning slides	428 (95%)

Results
At the date of assessment, 95% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

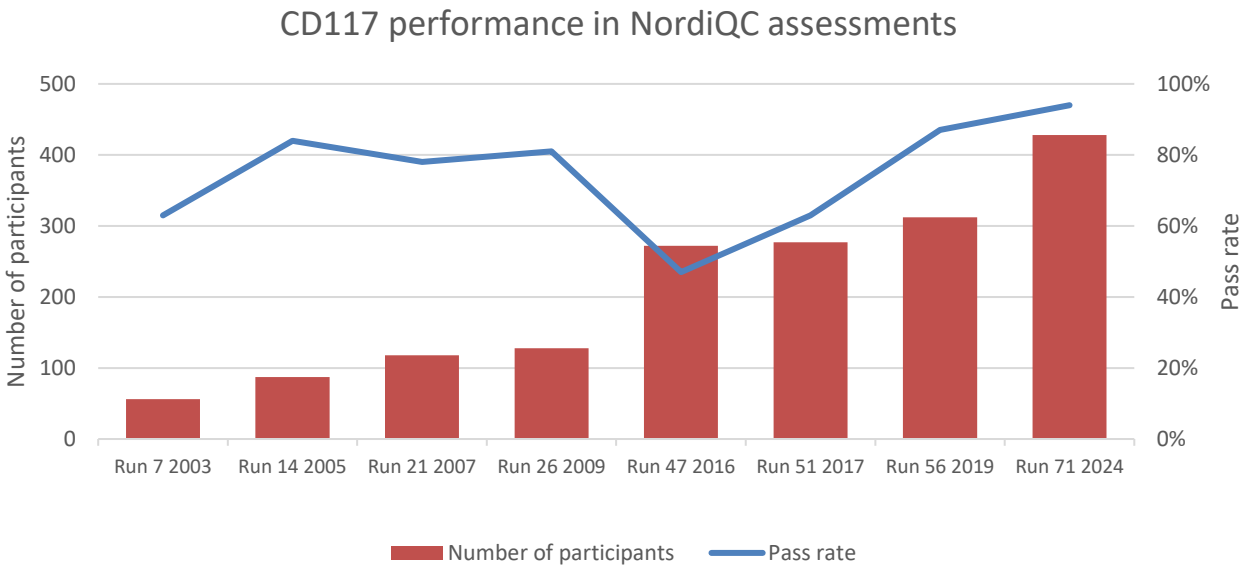
428 laboratories participated in this assessment. 94% achieved a sufficient mark (optimal or good), see Table 1a (page 2). Tables 1b and 1c summarize the antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:

- Too low concentration of the primary antibody
- Use of a less sensitive detection system.
- Heat Induced Epitope Retrieval (HIER) in citric based buffer.
- Inefficient HIER.
- Unexplained technical issues

Performance history
This was the 8th NordiQC assessment of CD117. The pass rate has steadily increased over the last four runs (see Graph 1).

Graph 1. **Proportion of sufficient results for CD117 in the eight NordiQC runs performed**



Controls
Appendix is recommended as positive and negative tissue control for CD117. The Cajal cells must show an at least moderate predominantly membranous staining reaction. Smooth muscle cells (muscularis propria and vascular structures) must be negative. Mast cells display a strong staining intensity and cannot be used as reliable control (both internal and external) to evaluate the reproducibility of the IHC assay, as these cells have a very high expression level of CD117. A weak to moderate staining reaction of neovascular structures (endothelium) and epithelial cells lining the basal compartment of the crypts should be expected by protocols providing a high level of analytical sensitivity for CD117.

Conclusion
The rmAb clones **YR145**, **EP10** and pAb **A4502** could all be used to obtain an optimal staining result for CD117. Although all three antibodies had a similar pass rate, the rmAb clones YR145 and EP10 had a significantly higher optimal rate compared to pAb A4502. Using one of these two rmAbs, either in a laboratory developed (LD) assay or in a Ready-to-use (RTU) format, the overall pass rate was 95% (244/256) and 81% (207/256) were assessed as optimal compared to 94% (147/156) sufficient and 58% (90/156) optimal for pAb A4502. Efficient HIER in an alkaline buffer, careful calibration of the primary Ab and preferrable use of a 3-step polymer/multimer detection system were the main prerequisites for optimal performance.
The RTU systems **790-7061** (Ventana/Roche) and **PA0007** (Leica Biosystems) based on the rmAb clone EP10 both provided a very high proportion of sufficient and optimal results. A pass rate of 100% was achieved with all protocol settings applying PA0007 (n=34) and all OptiView (760-700) based protocols (n=69) using 790-7061.

Table 1a. **Overall results for CD117, run 71**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR.[2]
Concentrated antibodies	240	152	70	15	3	93%	63%
Ready-To-Use antibodies	188	152	28	7	1	96%	81%
Total	428	304	98	22	4		
Proportion		71%	23%	5%	1%	94%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for CD117, Run 71**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **T595**	1	Leica Biosystems	0	0	0	1	-	-
rmAb clone **EP10**	10	Leica Biosystems	9	0	1	0	90%	90%
	6	Biocare Medical	2	3	1	0	83%	33%
	3	Epitomics	3	0	0	0	-	-
	2	Cell Marque	2	0	0	0	-	-
	1	Gene Tech	1	0	0	0	-	-
	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone **YR145**	45	Cell Marque	38	6	1	0	98%	84%
	2	Abcam	1	0	1	0	-	-
	2	Immunologic	1	0	0	1	-	-
	1	Biosite Histo	0	1	0	0	-	-
rmAb clone **BSR24**	2	Nordic Biosite	1	1	0	0	-	-
rmAb clone **IHC526**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **QR012**	1	Quartett	1	0	0	0	-	-
rmAb clone **ZR424**	1	Zeta Corporation	0	0	1	0	-	-
pAb **A4502**	156	Dako/Agilent	90	57	9	0	94%	58%
pAb **AB227749**	1	Abcam	0	1	0	0	-	-
pAb **RP063**	1	Diagnostic BioSystems	0	0	0	1	-	-
pAb **POC1152**	1	PathnSitu	1	0	0	0	-	-
pAb **503-1444**	1	Zytomed	0	1	0	0	-	-
pAb **BRB065**	1	Zytomed	0	0	1	0	-	-
Conc total	240		152	70	15	3		
Proportion			63%	29%	6%	1%	93%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).

Table 1c. **Ready-To-Use antibodies and assessment marks for CD117, Run 71**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR.[2]
rmAb clone **EP10 790-7061 (VRPS)[3]**	54	Ventana/Roche	42	10	2	0	96%	78%
rmAb clone **EP10 790-7061 (LMPS)[4]**	64	Ventana/Roche	52	9	3	0	95%	81%
rmAb clone **EP10 PA0007 (VRPS)[3]**	23	Leica Biosystems	23	1	0	0	100%	96%
rmAb clone **EP10 PA0007 (LMPS)[4]**	10	Leica Biosystems	9	1	0	0	100%	90%
rmAb clone **EP10 PME 296 AA**	1	Biocare Medical	1	0	0	0	-	-
rmAb clone **EP10 MAD-000644QD**	3	Master Diagnostica	2	1	0	0	-	-
rmAb clone **EP10 RMPD039**	1	Diagnostic BioSystems	0	0	1	0	-	-
rmAb clone **EP10 8267-C010**	3	Sakura Finetek	2	1	0	0	-	-
rmAb clone **YR145 117R**	21	Cell Marque	16	4	1	0	95%	76%
rmAb clone **YR145 Kit-0029**	1	Fuzhou Maixin	1	0	0	0	-	-
rmab clone **YR145 MON-RTU1058**	1	Monosan	1	0	0	0	-	-
rmAb clone **9.7 790-2951 (LMPS)[4]**	1	Ventana/Roche	0	1	0	0	-	-
rmAb clone **990G7E5 PA217**	1	Abcarta	1	0	0	0	-	-
rmAb clone **BP6064 I12222E-05**	1	Biolynx Biotechnology	1	0	0	0	-	-
rmAb clone **DA142 DMRD0177**	1	Dartmon Biotechnology	1	0	0	0	-	-
mAb clone **T595 AM423**	1	BioGenex	0	0	0	1	-	-
RTU total	188		152	28	7	1		
Proportion			81%	15%	4%	1%	96%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of CD117, Run 71
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
pAb **A4502**: Protocols with optimal results were based on HIER in an alkaline buffer using either Target Retrieval Solution (TRS) High pH (Dako/Agilent) (45/58), Cell Conditioning 1 (CC1, Ventana/Roche) (16/42), TRS High pH (3-in-1) (Dako/Agilent) (18/31) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (9/18) as retrieval buffer. The pAb was typically diluted in the range of 1:50-1:500 depending on the total sensitivity of the protocol employed. Using these protocol settings, 130 of 136 (96%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this buffer).

rmAb clone **YR145**: Protocols with optimal results were based on HIER using CC1 (Ventana/Roche) (26/31), TRS High pH (Dako/Agilent) (8/8), TRS High pH (3-in-1) (Dako/Agilent) (1/1), BERS2 (Leica Biosystems) (3/7) or Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:25-1:250 depending on the total sensitivity of the protocol employed. Using these protocol settings, 40 of 40 (100%) laboratories produced a sufficient staining result, 90% (36/40) optimal.

rmAb clone **EP10:** Protocols with optimal results were all based on HIER in an alkaline buffer using TRS High pH (Dako/Agilent) (8/8), CC1 (Ventana/Roche) (4/9), BERS2 (Leica Biosystems) (4/4), TRIS-EDTA/EGTA pH9 (1/1) or Dewax and HIER Buffer H (Epredia) (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:25-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 21 of 23 (91%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for CD117 for the most commonly used antibody concentrates on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
pAb **A4502**	18/30** (60%)	2/3	44/55 (80%)	0/1	14/37 (38%)	-	7/14 (50%)	0/2
rmAb clone **YR145**	-	-	8/8 (100%)	-	24/26 (92%)	-	3/5 (60%)	1/1
rmAb clone **EP10**	-	-	8/8 (100%)	-	4/9 (44%)	-	4/4	-

* 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).
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra plus
3) Bond III

Ready-To-Use antibodies
rmAb clone **YR145,** product no. **117R**, Cell Marque:
Protocols with optimal results were based on HIER using CC1, TRS High pH or BERS2 as retrieval buffer (efficient heating time 32-48, 30 or 20 min., respectively), 15-32 min. incubation of the primary Ab and OptiView (Roche/Ventana, 760-700), FLEX (Dako/Agilent, GV800/GV823) or Bond Refine (Leica Biosystems, DS9800) as detection system. Using these protocol settings, 11 of 11 (100%) laboratories produced an optimal staining result.

Ready-To-Use antibodies and corresponding systems
rmAb clone **EP10,** product no. **790-7061**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-64 min.) and 16-60 min. incubation of the primary Ab. Using these protocol settings together with OptiView 54/54 (100%) laboratories produced a sufficient staining result, 50/54 (93%) optimal and together with UltraView with or without Amplification Kit 42/46 (91%) participants passed, 32/46 (70%) scored as optimal.

rmAb clone **EP10,** product no. **PA0007,** Leica Biosystems Bond III/Prime:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20 min.) and 15-30 min. incubation of the primary Ab together with Bond detection system (DS9800, DS9284). Using these protocol settings, 26/26 (100%) laboratories produced a sufficient staining, 25/26 (96%) optimal.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for CD117 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark rmAb clone **EP10, 790-7061, UltraView**	94% (34/36)	75% (27/36)	83% (10/12)	42% (5/12)
Ventana Benchmark rmAb clone **EP10, 790-7061, OptiView**	100% (18/18)	83% (15/18)	100% (47/47)	91% (43/47)
Leica Bond rmAb clone **EP10, PA0007**	100% (24/24)	96% (23/24)	100% (8/8)	88% (7/8)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment and in concordance with the previous NordiQC CD117 assessments, the prevalent feature of an insufficient staining result was a too weak or false negative staining reaction of cells expected to be demonstrated. This pattern was observed in 65% (17/26) of the insufficient results. Excessive background or false positive reaction was seen in 23% (6/26) of the insufficient results (see Fig. 6a). The remaining insufficient results were characterized by completely missing hematoxylin-counterstaining compromising the interpretation. An aberrant nuclear staining reaction (see Fig. 6b) was seen in eight participant slides stained with different primary antibody clones. When presented as a weak staining together with an otherwise optimal result, the slides were downgraded to good as it is a separate compartment from what is expected to be positive. Virtually all laboratories were able to demonstrate CD117 in high-level antigen expressing structures such as neoplastic cells of the GISTs and mast cells (all tissue cores). Demonstration of CD117 in low-expressors such as Cajal cells in the appendiceal muscularis propria and neovascular structures were more challenging and required a carefully calibrated protocol.

The **pAb A4502** remained as the most widely used antibody for the demonstration of CD117, utilized by 36% (156/428) of participants. Used as a concentrate within a laboratory developed (LD) assay, pAb A4502 gave an overall pass rate of 94% (147/156) (see Table 1b) which is a significant increase from previous NordiQC assessment runs (52% in run 51, 2017, and 82% in run 56, 2019). The proportion of sufficient results was similar on all four main IHC platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems, being in the range of 89-97%, however the proportion of optimal results varied on the IHC platforms, being highest on Dako Omnis (46/61, 75%) and lowest on Ventana Benchmark (16/31, 49%). Protocols providing optimal results were typically based on HIER in an alkaline buffer e.g. TRS High pH, BERS2 or CC1 with an incubation time of ≥20 min. for Dako/Agilent and Leica Biosystems platforms and ≥32 min. for Ventana Benchmark platforms. Both 2- and 3-layered detection systems could be used for achieving an optimal result.

Within LD assays based on a primary antibody concentrate, the two rmAb clones **YR145** and **EP10** provided high pass rates of 94% (48/51) and 91% (21/23), respectively, similar to the performance of pAb A4502. The proportion of optimal results was 78% (58/74) for both rmAb clones which is higher compared to 58% (90/156) obtained with the pAb A4502 (see Table 1b). For both rmAb clones YR145 and EP10, efficient HIER in an alkaline buffer, sufficient primary antibody concentration and preferably the use of a sensitive 3-step multimer/polymer detection system provided high proportions of sufficient and optimal results. The mean dilution factor across the two clones amongst all optimal results (n=57) was the highest, being 1:110 (excluding one lab who used the primary Ab concentration of 1:1800), while all assays scored as Good had a mean dilution factor of 1:181 (n=11) and for insufficient results, the mean dilution factor was 1:240 (n=5). Within the main fully automated staining platforms, all slides stained with rmAb clones EP10 and YR145 on Dako Omnis were scored as optimal (n=17). For rmAb clone EP10, all slides stained on Leica Bond III were also scored as optimal (n=4), while the performance on the Ventana Benchmark stainer platforms was slightly inferior with a pass rate of 78% (7/9) achieved, 44% (4/9) optimal. Although dataset is very small, 80% (4/5) of slides not receiving an optimal mark on the Ventana platforms with rmAb clone EP10 were using the primary Ab from Biocare Medical, which otherwise got optimal results on the Dako Omnis and Leica Bond, despite comparable protocol settings being applied on all three IHC platforms. The rmAb clone YR145 performed better on the Ventana Benchmark platforms,

achieving a pass rate or 97% (32/33), 82% (27/33) optimal, compared to 88% (7/8) sufficient and 50% (4/8) optimal results on the Leica Bond. The moderate level of optimal results on Leica Bond platforms was mostly related to the dilution factors applied, as all optimal protocols were based on antibody concentration of 1:50 – 1:100, whereas all other protocols used a dilution factor of 1:150 or more.

The new RTU system **790-7061** from Ventana/Roche based on the rmAb clone **EP10** has been widely implemented and served as the second most utilized primary antibody product used by 28% (118/428) of laboratories. The RTU system provided a very high pass rate of 96% (113/118) (see Table 1c). As shown in Table 3, all participants using the product on the intended platforms together with a sensitive OptiView detection system were assessed as sufficient, 89% (58/65) optimal. Vendor recommended protocol settings (VRPS) for both UltraView and OptiView generally produced sufficient results, as the only two insufficient results were due to the lack of counterstaining. The most successful protocol modifications were based on prolonging HIER and/or primary Ab incubation when using OptiView increasing the overall analytical sensitivity of the IHC assay and still with preserved specificity.

As in the previous runs, the RTU system from Leica Biosystems, **PA0007** based on the rmAb clone **EP10** demonstrated superior performance for detection of CD117, providing a pass rate of 100% (n=34) of which 94% (32/34) of the protocols were assessed as optimal. Both vendor and laboratory modified protocol settings could be used to obtain optimal results (see Table 3).

The RTU format **117R** based on rmAb clone **YR145** from Cell Marque has remained as one of the relatively widely used RTU products, utilized by 5% (21/428) of all participants and achieving a high pass rate of 95% (20/21), 76% (16/21) optimal. All 4 slides stained on the Dako Omnis were assessed as optimal (protocol settings: HIER in TRS High pH 30 min., Ab incubation 15-30 min., FLEX or FLEX+ as detection system). The majority of participants applied the RTU product on Ventana Benchmark stainer platforms. All slides scored as optimal were subjected to HIER in CC1 for ≥32 min., primary Ab incubation was generally 32 min. (9/11) and a 3-step detection system as OptiView or UltraView with Amplification Kit was used (one participant used OptiView with OptiView Amplification Kit).

This was the eighth assessment of CD117 in NordiQC (see Graph 1). The proportion of sufficient results was 94%, which is an improvement from 87% achieved in the previous run 56 in 2019 and overall the highest pass rate for CD117 to date. Similarly to the previous run, the main parameters contributing to the positive development were the extended use of robust primary rmAbs (YR145 and EP10) more than doubling from 119 (of 312, 38%) in the past assessment to 245 (of 428, 57%) in this assessment on the expense of the less successful abs as pAb 4502 and especially the rmAb clone 9.7. Throughout the different assessment runs a harmonization of the protocol settings has been observed. While previously omission of HIER or HIER in low pH buffers were frequently applied, now virtually all protocols are based on HIER in high pH buffers. The latter, in part, also contributed to the higher pass rate of the most commonly used pAb A4502, having a significant impact on the general pass rate, however still exhibiting a lower optimal rate compared to the relatively robust rmAbs EP10 and YR145. Lastly, Ventana Benchmark users now have a successful RTU system 790-7061 based on rmAb clone EP10 showing superior results compared to the former RTU system 790-2951 based on rmAb clone 9.7 that in the past assessments proved to perform inferiorly. Although some clones seem to be relatively more robust, it is important to conclude that sufficient HIER and concentration of the primary antibody together with a sensitive 3-step detection system is an important prerequisite for an optimal CD117 staining reaction.

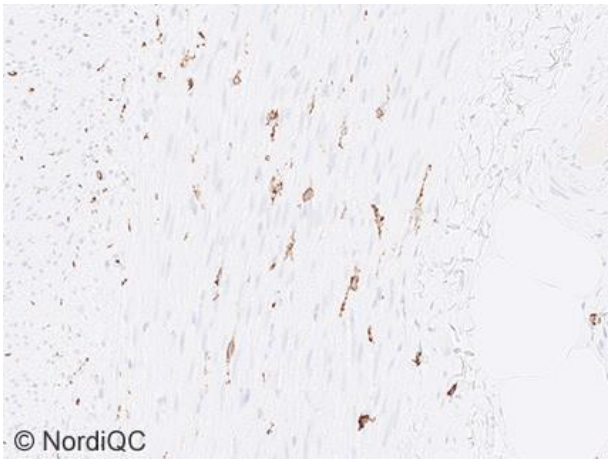


Fig. 1a
Optimal CD117 staining reaction of the appendix using the Ventana/Roche RTU system 790-7061 based on rmAb clone EP10 as RTU on the Ultra Plus platform and applying vendor recommended protocol settings with HIER for 32 min. in CC1, 16 min. Ab incubation and OptiView as detection system - same protocol used in Figs. 2a - 5a. Virtually all Cajal cells in the appendiceal muscularis propria are distinctively stained with moderate intensity - compare with Fig. 1b. The smooth muscle cells are unstained.

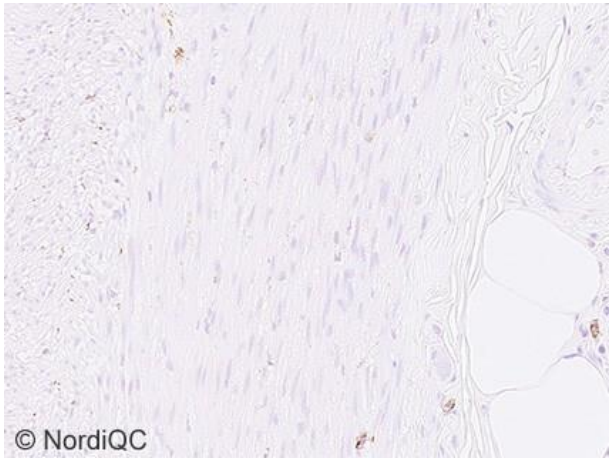


Fig. 1b
Insufficient CD117 staining reaction of the appendix using the polyclonal Ab A4502 on the Dako Omnis platform without any HIER and a relatively high dilution factor of 1:100 together with the 2-layered detection system EnVision FLEX – same protocol used in Figs. 2b – 5b. The proportion of Cajal cells are significantly reduced, only displaying a faint to weak staining intensity - compare with Fig. 1a.

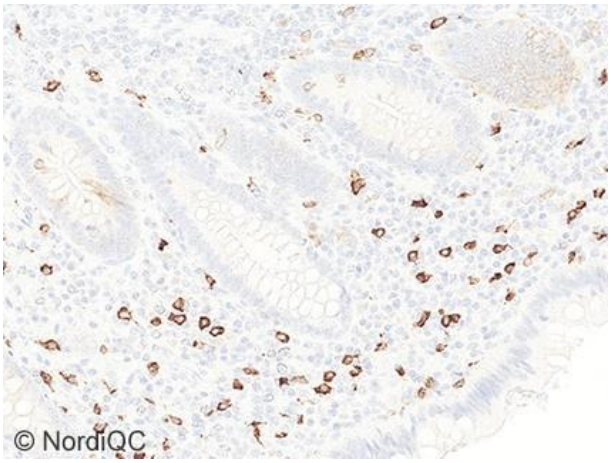


Fig. 2a
Optimal CD117 staining reaction of the appendix using same protocol as in Fig. 1a. Dispersed epithelial cells of the crypts show a weak to moderate and distinct staining reaction while mast cells exhibit a strong staining reaction.

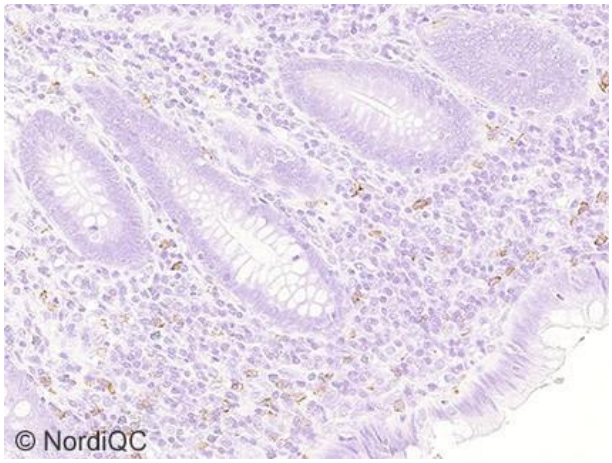


Fig. 2b
Insufficient CD117 staining reaction of the appendix using same protocol as in Fig. 1b. No staining reaction in the epithelial cells in the appendiceal crypts can be seen. Mast cells are visible, however with a significantly lower expression intensity - compare with Fig. 2a, same area.

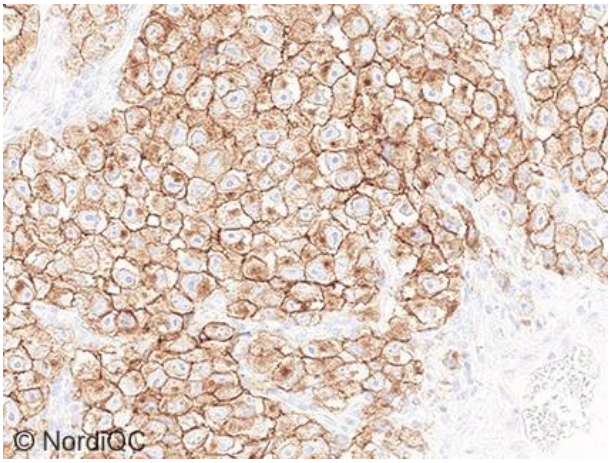


Fig. 3a
Optimal CD117 staining reaction of the seminoma, tissue core no. 2, using same protocol settings as in Figs. 1a – 2a. Virtually all neoplastic cells show a moderate to strong, predominantly membranous staining reaction.

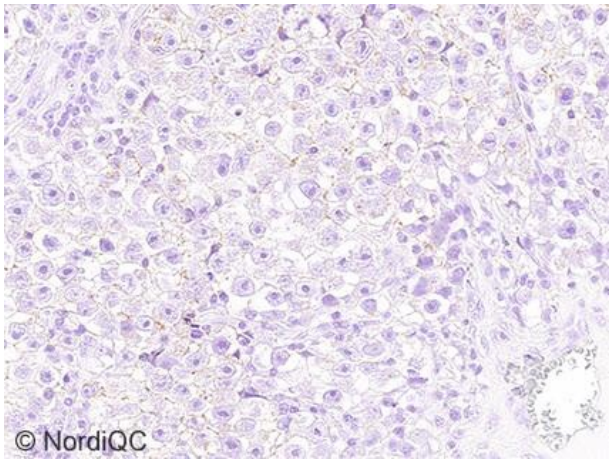


Fig. 3b
Insufficient CD117 staining reaction of the seminoma, tissue core no. 2, using same protocol settings as in Figs. 1b – 2b. Only a proportion of scattered neoplastic cells show a faint, barely perceptible membranous staining reaction - compare with Fig. 3a, same area.

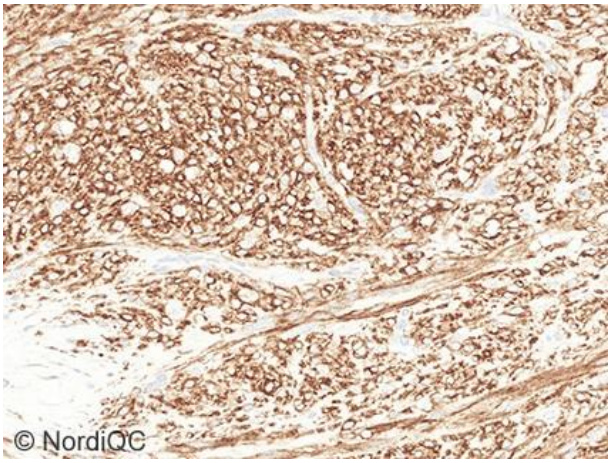


Fig. 4a
Optimal CD117 staining reaction of the GIST, tissue core no. 3, using same protocol settings as in Figs. 1a-3a. All the neoplastic cells show a distinct strong staining reaction.

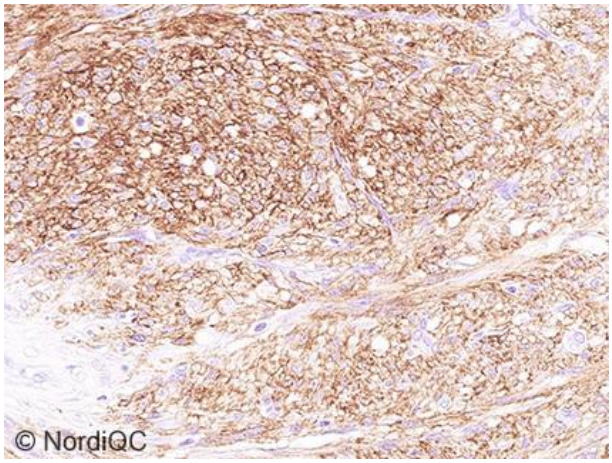


Fig. 4b
Staining reaction for CD117 of the GIST, tissue core no. 3, using the same protocol as in Figs. 1b-3b. All neoplastic cells show a moderate to strong staining reaction - compare to Fig. 4a. Although a significant decrease in staining intensity was seen in other tissues (see Figs. 1b-3b), the high CD117 antigen level in GIST ensures an at least moderate staining reaction with an otherwise insufficient protocol.

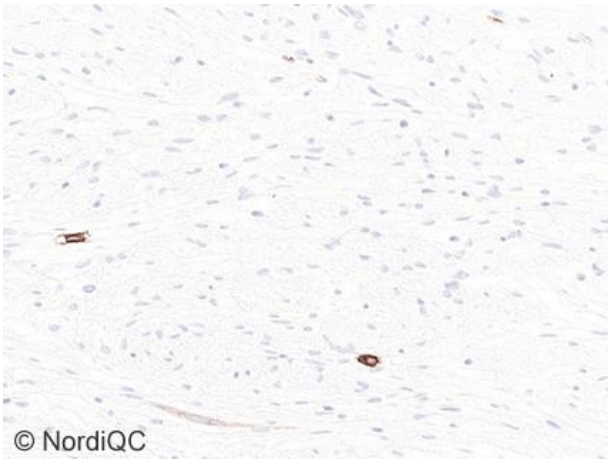


Fig. 5a
Optimal CD117 staining reaction of the schwannoma, tissue core no. 5, using same protocol settings as in Figs. 1a-4a. All the neoplastic cells are negative, while mast cells exhibit a strong staining reaction and neovascular structures a weak staining reaction.

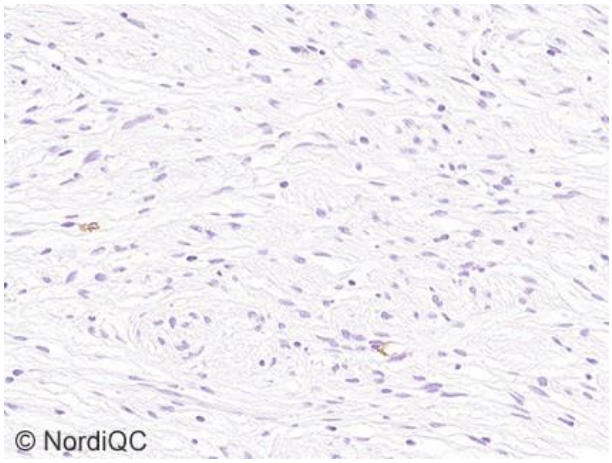


Fig. 5b
Insufficient CD117 staining reaction of the schwannoma, tissue core no. 5, using same protocol settings as in Figs. 1b-4b. Both the neoplastic cells and neovascular structures are completely negative, while mast cells retain a weak staining reaction.

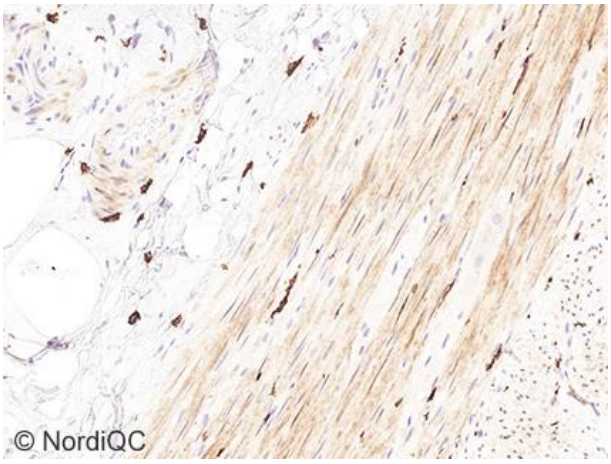


Fig. 6a
Insufficient CD117 staining reaction of the appendix using the rmAb clone YR145 from Abcam within a LD assay on the Leica Bond III instrument. Smooth muscle cells in appendiceal lamina muscularis propria and vessel walls are aberrantly labelled (false positive) compromising the interpretation.

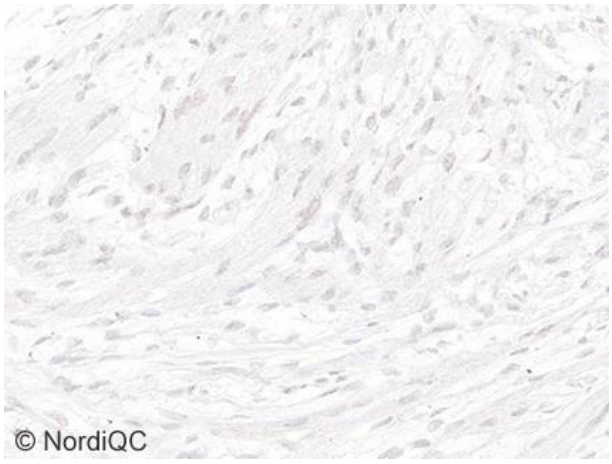


Fig. 6b
Staining reaction assessed as good for CD117 of the schwannoma, tissue core no. 5, using pAb A4502 (diluted 1:200) on the Ventana Ultra platform. A weak aberrant nuclear staining reaction can be seen in scattered neoplastic cells. However, the interpretation is not completely hindered as the incorrect staining is in a different cellular compartment.

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