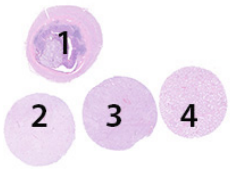
Assessment Run 56 2019

CD117

Material
The slide to be stained for CD117 comprised:



1. Appendix, 2. Desmoid tumor, 3. Gastrointestinal stromal tumor (GIST),
4. Testicular germ cell neoplasia in situ (GCNIS).

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 strong, distinct staining reaction of virtual all neoplastic cells in the GIST.
- A strong, distinct membranous staining reaction of all neoplastic cells in the GCNIS.
- A strong, predominantly membranous staining reaction of mast cells in all specimens.
- A weak to moderate, distinct staining reaction of neovascular endothelial structures (all cores) and of scattered epithelial cells lining the basal compartment of crypts in the appendix.
- No staining reaction of smooth muscle cells (all specimens).
- The desmoid tumour was excluded in the assessment due to a technical issue (see comments).

Participation

Number of laboratories registered for CD117, run 56	321
Number of laboratories returning slides	312 (97%)

Results
312 laboratories participated in this assessment. 271 (87%) achieved a sufficient mark (optimal or good). Table 1 summarizes the used antibodies (Abs) and the assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low or too high concentration of the primary antibody
- Less successful primary antibodies - pAb A4502 (Dako) and rmAb 9.7 (Ventana/Roche)
- Heat Induced Epitope Retrieval (HIER) in citric based buffer
- Inefficient HIER (too short efficient heating time or too low temperature)
- Less sensitive detection systems
- Unexplained technical issues

Performance history
This was the seventh NordiQC assessment of CD117. The pass rate increased significantly compared to the result obtained in run 51, 2017 (see Table 2).

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

	Run 7 2003	Run 14 2005	Run 21 2007	Run 26 2009	Run 47 2016	Run 51 2017	Run 56 2019
Participants, n=	56	87	118	128	272	277	312
Sufficient results	63%	84%	78%	81%	47%	63%	87%

Conclusion
The rmAb clones YR145 and EP10 were the most successful antibodies for immunohistochemical demonstration of CD117. Using one of these two Abs, either within a laboratory developed (LD) IHC assay or IHC system based on a Ready-to-use (RTU) format, the overall pass rate was 97% of which 78% were assessed as optimal. Efficient HIER, preferable in an alkaline buffer, careful calibration of the primary Ab and use of a 3-step polymer/multimer detection system were the main prerequisite for optimal performance. The RTU system from Leica, PA0007 based on the rmAb EP10 demonstrated a superior performance for CD117, providing a pass rate of 100% and 94% optimal marks.
Optimal results could also be obtained with the mAb MX041, pAb A4502 and the RTU format based on the rmAb 9.7. Assays based on the pAb A4502 (Dako) were used by the majority of the participants (47%),

providing a pass rate of 82% but with a significant lower proportion of optimal results (27%) compared to the rmAbs YR145 and EP10. A similar pattern was seen with the RTU system based on the rmAb 9.7 (Ventana), where 78% were assessed as sufficient and 38% were scored as optimal.
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 typically display a strong staining intensity and cannot be used as a reliable and critical positive control for CD117 (both internal and external) to monitor the required level of analytical sensitivity of the assay, as these cells only display 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 optimal results.

Table 1. **Antibodies and assessment marks for CD117, run 56**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
pAb **A4502**	146	Dako/Agilent	40	80	22	4	82%	86%
pAb **61-0020**	1	GeneMed	0	1	0	0	-	-
pAb **RP063-05**	1	Diagnostic BioSystems	0	1	0	0	-	-
pAb **E1440**	1	Spring Bioscience	0	1	0	0	-	-
rmAb clone **YR145**	39 7 2 2 1 1	Cell Marque Nordic Biosite Immunologic Epitomics Abcam US Biological	44	4	4	0	92%	98%
rmAb clone **EP10**	8 6 5 2 1 1	Biocare Medical Epitomics Leica/Novocastra Nordic Biosite Diagnostic Biosystem PathnSitu Biotechnologies	16	7	0	0	100%	100%
mAb clone **T595**	2	Leica/Novocastra	0	0	2	0	-	-
Ready-To-Use antibodies								
rmAb clone **9.7 790-2951**	40	Ventana/Roche	15	16	8	1	78%	93%
rmAb clone **YR145 117R-10-ASR**	20	Cell Marque	14	6	0	0	100%	100%
rmAb clone **YR145 AN465-5M**	1	Biogenex	0	1	0	0	-	-
rmAb clone **YR145 44218**	1	Menarini Diagnostics	1	0	0	0	-	-
rmAb clone **EP10 PA0007**	16	Leica Biosystems	15	1	0	0	100%	100%
rmAb clone **EP10 MAD-000644QD**	2	Master Diagnostica	0	2	0	0	-	-
rmAb clone **EP10 PME-296**	2	Biocare Medical	1	1	0	0	-	-
rmAb clone **EP10 8267-C010**	2	Sakura Finetek USA	2	0	0	0	-	-
mAb clone **MX041 MAB-0745**	1	Maixin	1	0	0	0		
pAb **PDR045**	1	Diagnostic Biosystems	0	1	0	0	-	-
Total	312		149	122	36	5	-	
Proportion			48%	39%	12%	2%	87%	

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 56
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
pAb **A4502**: Protocols with optimal results were all based on HIER in an alkaline buffer using either Cell Conditioning 1 (CC1, Ventana) (14/56) *, Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9, Dako) (21/65), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (4/13) or Novocastra™ Epitope Retrieval Solutions pH 9 (Leica) (1/1) 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, 92 of 107 (86%) 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 based on HIER using CC1 (Ventana) (30/35), TRS high pH 9 (3-in-1)(Dako) (11/11), TRS high pH 9 (Dako/Agilent) (1/1), BERS2 (Leica) (1/3) or Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:250 depending on the total sensitivity of the protocol employed. Using these protocol settings, 41 of 42 (98%) laboratories produced a sufficient staining.

rmAb clone **EP10:** Protocols with optimal results were all based on HIER in an alkaline buffer using either CC1 (Ventana) (5/8), TRS high pH 9 (3-in-1)(Dako) (5/6), BERS2 (Leica) (3/4) or TRIS-EDTA/EGTA pH9 (3/ 4) 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, 22 of 22 (100%) laboratories produced a sufficient staining.

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

Concentrated antibodies	**Dako Autostainer Link/Classic**		**Dako Omnis**		**Ventana BenchMark GX /XT/ Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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**	9/30** (30%)	0/1	9/24 (38%)	0/1	14/42 (33%)	0/0	4/11 (36%)	0/1
rmAb clone **YR145**	100% (5/5)	-	100% (6/6)	-	24/28 (86%)	-	1/2	1/1
rmAb clone **EP10**	-	-	100% (5/5)	-	63% (5/8)	-	3/4	-

* 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)

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 BERS2 (efficient heating time 20 min.) and 15-30 min. incubation of the primary Ab. Bond Refine (DS9800) was used as detection system. Using these protocol settings, 15 of 15 (100%) laboratories produced a sufficient staining. One protocol obtaining an optimal result was based on no pre-treatment at all.

rmAb clone **9.7,** product no. **790-2951,** Roche/Ventana, Benchmark XT/Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient HIER time for 64 min. at 94-100°C), 28-60 min. incubation time of primary Ab and OptiView (Ventana, 760-700) with or without amplification (Ventana, 760-099/ 4-16 min. incubation at 36°C) as detection system. Using these protocol settings, 14 of 15 (93%) laboratories produced a sufficient staining. One protocol obtaining an optimal result was based on no pre-treatment at all.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). The performance of the individual assays were evaluated as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations but also as laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **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

Leica BOND MAX/III rm EP10 **PA0007**	100% (10/10)	100% (10/10)	100% (6/6)	83% (5/6)
VMS Ultra/XT rm 9.7 **790-2951**	(0/1)	(0/1)	79% (31/39)	38% (15/39)

* 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 >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

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 61% of the insufficient results (25 of 41). Too weak staining result in combination with either background and/or false positive reaction was seen in 12% of the insufficient results (5 of 41). The remaining insufficient results were characterized by poor signal-to-noise ratio and/or false positive staining reaction of smooth muscle cells (especially lamina muscularis propria and arterial walls in the appendix) compromising the interpretation.
Virtually all laboratories were able to demonstrate CD117 in high-level antigen expressing structures such as neoplastic cells of the GIST and mast cells (all tissue cores). Demonstration of CD117 in low-level antigen expressing structures as Cajal cells in the appendiceal muscularis propria was more challenging and required a carefully calibrated protocol.
The desmoid tumor was excluded from assessment due to an aberrant faint to weak staining reaction of the neoplastic cells. This staining pattern was seen with virtual all antibody formats including the rmAbs YR145 and EP10 that previously have not shown this staining reaction.

The pAb A4502 was the most widely used antibody for the demonstration of CD117. Used as a concentrate within a Laboratory Developed (LD) assay, pAb A4502 gave an overall pass rate of 82% (120 of 146) (see Table 1). As shown in Table 3, optimal results could be obtained on all four main IHC platforms from Dako, Leica and Ventana, although the proportion of optimal scores was lower compared to the rmAbs. If staining was performed on the Omnis, applying optimal protocol settings, using HIER in TRS pH9 and detection kits as provided by the vendor (Dako), 38% (9/24) of the protocols were assessed as optimal. In comparison and applying similar protocols settings as for the pAb4502 on the Omnis, 100% (11/11) of the protocols based on the rmAbs YR145 and EP10 were scored as optimal. Same pattern was seen on other platforms e.g. on Benchmark XT/Ultra (Ventana) (see Table 3).
Protocols providing optimal results were all based on HIER in an alkaline buffer e.g. BERS2 (Leica) or CC1 (Ventana). Five protocols were based on HIER in acidic buffer and none was assessed as optimal. As observed in the previous run 51, no single parameters could be identified discriminating between sufficient and insufficient staining results (e.g. dilution factor of the primary Ab or the use of a 2-step versus 3-step multimer/polymer detection system). However, the proportion of optimal results was higher if efficient HIER in alkaline buffer was performed (≥ 20 min. at 95-100°C) and a 3 -step polymer/multimer detection system was applied. If pAb A4502 was used within a LD assay on the Dako Autostainer, only 13 % (1/8) of the laboratories obtained an optimal mark if the protocols were based on short HIER time (≤ 20 min at 95-100°C). In contrast, and if HIER time was prolonged (≥ 20 min at 95-100°C), proportion of optimal results increased to 28% (9/32). The choice of detection system with different analytical sensitivity also influenced the number of optimal results. Using optimal protocol settings for the pAb A4502 on the Benchmark platform (Ventana) (see Table 3), 86% (12/14) of the laboratories applying OptiView (+/- amplification) obtained an optimal staining result. In the remaining protocols assessed either as good or insufficient (borderline or poor), only 29% (8/28) of the laboratories used OptiView (+/- amplification) and in this group, the vast majority of protocols were based on Ultraview (+/- amplification) as detection system, typically providing weaker staining intensity and/or background problems. Of all protocols assessed as insufficient (see Table 1), 68% (17/25) of the participants applied a less sensitive 2-step polymer/multimer detection system. One laboratory used the biotin-based detection system iView (Ventana) and was assessed as borderline due to false positive reactions.

In this run, and used within a LD assay, the two rmAbs clone YR145 and EP10 provided significantly higher pass rates and proportion of optimal results compared to the performance of pAb A4502. The number of laboratories using one of these two clones is still increasing, and grouped together, the overall pass rate was 95% (71 of 75). The proportion of optimal results was 80% (60 of 75) and significantly higher compared to 27% (40 of 146) obtained with the pAb A4502 (see Table 1). For both rmAbs (YR145 and EP10), efficient HIER (preferable in an alkaline buffer) and the use of a sensitive 3-step multimer/polymer detection system (e.g. Flex+ (Dako/Agilent) and Optiview (Ventana/Roche)) provided high proportions of sufficient results. Using these conditions, and in the optimal dilution range of 1:25-1:250 of the primary Abs, 100% (38/38) of the protocols were assessed as sufficient and with an impressive level of 92% (35 of

38) marked as optimal. For the four protocols that were assessed as insufficient, one laboratory used too low concentration of the primary in combination with too short incubation time and for the remaining laboratories, no technical parameters could be identified explaining for the insufficient performance.

In this assessment, the RTU system from Ventana (790-2951) based on the rmAb 9.7 provided a pass rate of 78% (31/40) (see Table 1). As shown in Table 4, only one laboratory (1 of 40) followed the official protocol recommendation from the vendor and obtained an insufficient result. Recommeneded protocol settings are based on HIER in CC1 (60 min. at 100°C), 32 min. incubation in primary ab (at 42°C) and iView as the detection system and performed on a Benchmark XT platform. In general, a limited number of participants used iView or the Benchmark XT platform (1 and 4 laboratories, respectively) and thus the package insert needs to be updated meeting the requirements of present applied reagents (e.g. OptiView) and the far more common platform Benchmark Ultra. Noteworthy, nearly all laboratories (39 of 40) used modified protocol settings (see Table 4). In run 51, only one laboratory obtained an optimal result and the protocol was based on HIER using CC1 (efficient HIER time for 64 min. at 100°C), 60 min. incubation time of primary Ab and OptiView (Ventana, 760-700) with amplification (Ventana, 760-099/ 16 min. incubation at 36°C) as detection system. In this assessment, 67% (10/15) of the laboratories obtaining an optimal mark applied similar protocol settings with minor adjustment of incubation time of primary Ab (majority of laboratories used 60 min.) and time in amplification reagents (majority of laboratories used 8+8 min). Using optimal protocol settings as described above, the pass rate increased to 93% indicating that the assay might provide appropriate analytical sensitivity and specificity as long as correctly modified protocol parameters are applied.

According to the package insert of the RTU format from Cell Marque (117R-10-ASR) based on the rmAb YR145, the product is not designed for a specific system/platform. However, the vast majority of participants (18/20) applied this format on Benchmark Ultra/XT platforms (Ventana) and provided a high proportion of sufficient and optimal results. Using HIER in CC1 (efficient HIER time for 32-64 min. at 95-100°C), 24-72 min. incubation of the primary Ab at 36 °C and UltraView (760-500, Ventana) with amplification (760-080, Ventana) or OptiView with or without amplification (760-099, Ventana) as the detection systems, 100% (15/15) of the protocols were assessed as sufficient and 73% (11/15) were optimal.

As in the previous run, the RTU system from Leica, PA0007 based on the rmAb EP10 demonstrated superior performance for detection of CD117, providing a pass rate of 100% (16/16) of which 94% (15/16) of the protocols were assessed as optimal. Both vendor and laboratory modified protocol settings could be used to obtain optimal results (see Table 4).

This was the seventh assessment of CD117 in NordiQC (see Table 2). A pass rate of 87% was obtained, which is a significant improvement compared to the former Run 51, 2017. The following parameters contributed to the positive development: 1) The use of robust primary rmAbs (YR145 and EP10) has increased from 29% (80 of 277) in the previous run to 38% (119 of 312) in this assessment, 2) The number of laboratories using a pAb, which seems less efficient for demonstration of CD117 compared to the rmAbs, has been reduced from 59% (164 of 277) in the previous run to 48% (149 of 312) in this assessment, 3) The proportion of laboratories following advices given by NordiQC in the past runs, typically recommending HIER in an alkaline buffer, careful calibration of the primary Ab and a 3-step multimer/polymer detection system, has increased, 4) Less challenging tissue circulated to the participants. Importantly, protocols must stain according to the expected and clinical relevant antigen levels as characterized by the reaction patterns in the recommended control material (see below).

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.

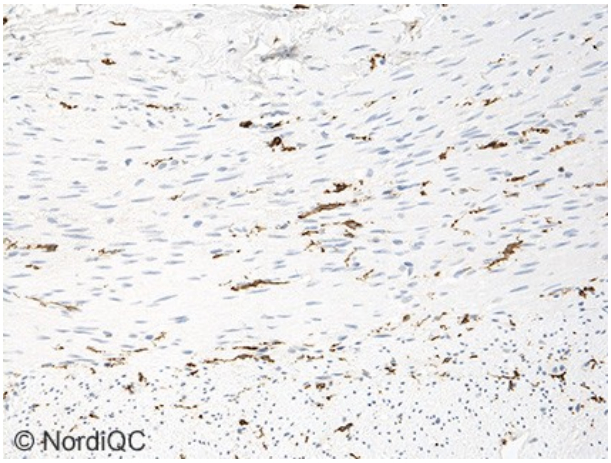


Fig. 1a (x200)
Optimal CD117 staining of the appendix using the rmAb YR145 optimally calibrated, HIER in an alkaline buffer (BERS2, Leica) and Bond Refine (Leica) as detection system - same protocol used in Figs. 2a - 3a. Virtually all Cajal cells in the appendiceal muscularis propria are distinctively stained with strong intensity - compare with Fig. 1b. The smooth muscle cells are unstained.

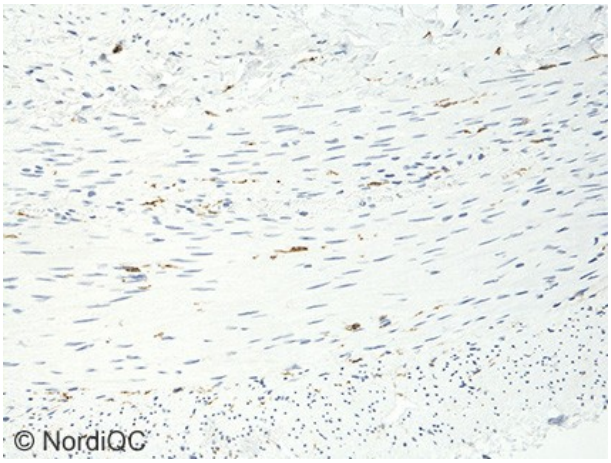


Fig. 1b (x200)
Insufficient CD117 staining of the appendix using the rmAb clone YR145, too diluted and too short incubation time, with same protocol settings as the protocol in Fig 1a – same protocol used in Figs. 2b – 3b. The proportion of Cajal cells are significantly reduced, only displaying a faint to weak staining intensity - compare with Fig. 1a.

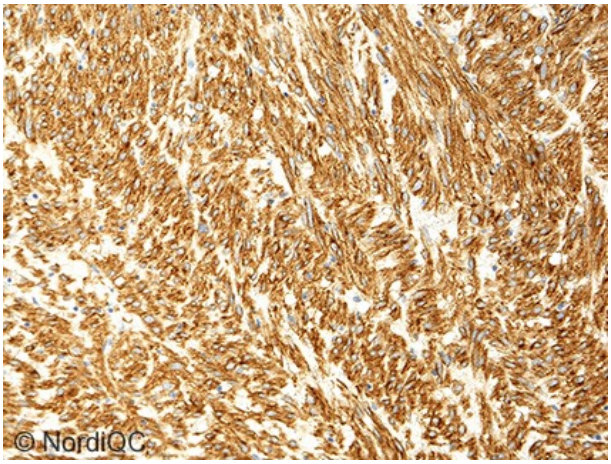


Fig. 2a (x200)
Optimal CD117 staining of the GIST using same protocol as in Fig. 1a. All the neoplastic cells show a strong but distinct staining reaction.

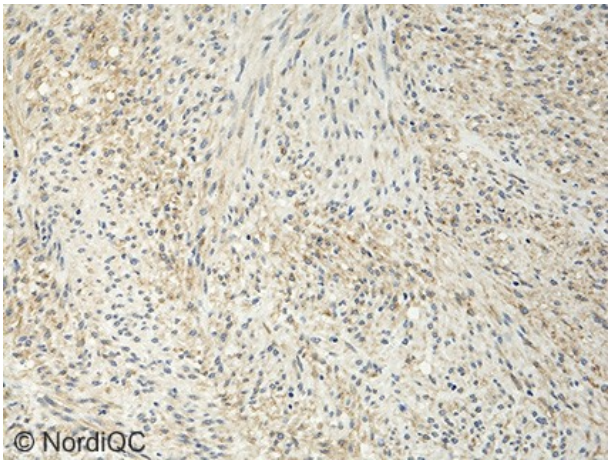


Fig. 2b (x200)
Insufficient CD117 staining of the GIST using same protocol as in Fig. 1b. The neoplastic cells are completely negative or only faintly demonstrated - compare with Fig. 2a.

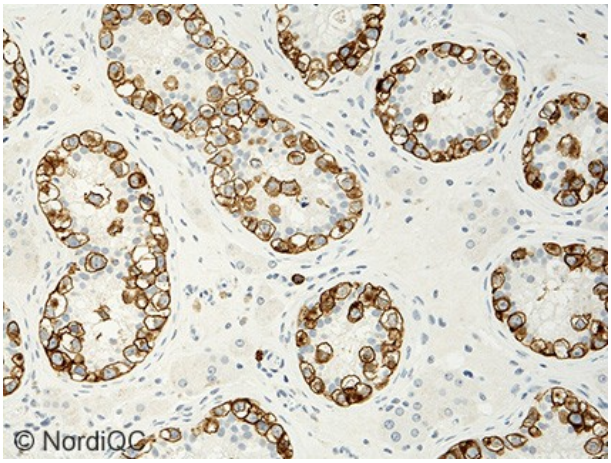


Fig. 3a (x200)
Optimal CD117 staining of the GCNIS using same protocol as in Fig. 1a - 2a. All intratubular neoplastic cells in the testis displays a strong, distinct membranous staining reaction.

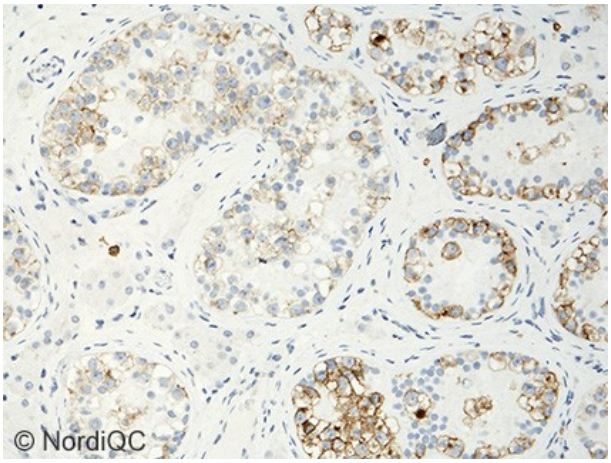


Fig. 3b (x200)
Insufficient CD117 staining of the GCNIS using same protocol as in Fig. 1b - 2b. The proportion of stained intratubular neoplastic cells in the testis is reduced and intensity is too weak - compare with Fig. 3a.

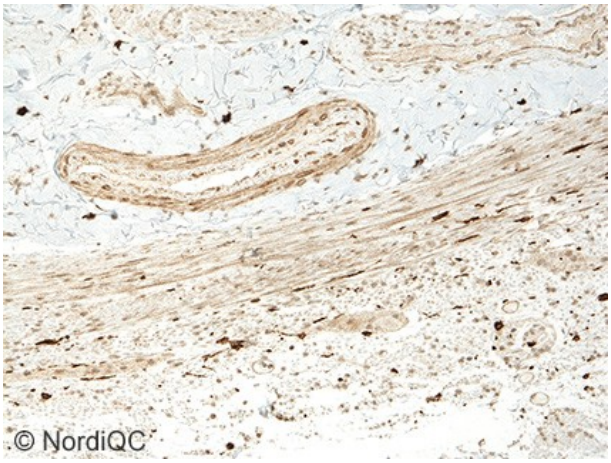


Fig. 4a (x200)
Insufficient CD117 staining of the appendix using the pAb A4502 within a LD assay, with a too high concentration, HIER in an alkaline buffer (CC1, Ventana) and UltraView with amplification (Ventana) as detection system. Smooth muscle cells (appendiceal lamina muscularis proria and arterial walls) are aberrantly labelled (false positive) compromising interpretation of the specific staining of the Cajal cells.

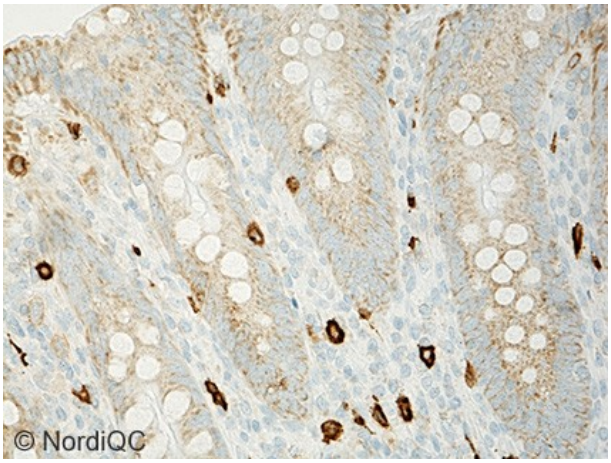


Fig. 4b (x200)
Insufficient CD117 staining of the appendix using the rmAb YR145 within a LD assay, HIER in an alkaline buffer (CC1, Ventana) and iView (Ventana) as detection system. The mast cells in lamina proria mucosa show the expected expression level for CD117, whereas the epithelial cells are aberrantly stained due to endogenous biotin displaying a granular expression pattern. Detection systems based on biotin should be avoided due to low sensitivity and risk of false positive staining result. At least, and using these systems, laboratories should incorporate a blocking procedure for endogenous biotin.

MB/LE/RR/MV/SN 08.07.2019