## Material
The slide to be stained for CD117 comprised:

1. Desmoid tumour, 2. Appendix, 3-4. Gastrointestinal stromal tumour (GIST),
5. 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:

- 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 all neoplastic cells in the GIST, tissue core no. 3.
- A strong, distinct membranous staining reaction of neoplastic cells in the GCNIS.
- An at least weak to moderate, distinct staining reaction of virtually all neoplastic cells in the GIST, tissue core no. 4.
- 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 epithelial cells lining the basal compartment of crypts in the appendix.
- No staining reaction of smooth muscle cells (all specimens) and neoplastic cells in the desmoid tumour.
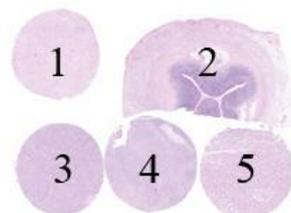
## Participation

| | |
|---|---|
| Number of laboratories registered for CD117, run 51 | 295 |
| Number of laboratories returning slides | 277 (94%) |

## Results
277 laboratories participated in this assessment. 176 (63%) 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)
- Omission of HIER (protocols based on the pAb A4502)
- Less sensitive detection systems (protocols based on the rmAb YR145 and rmAb EP10)
- Unexplained technical issues

## Performance history
This was the sixth NordiQC assessment of CD117. The overall pass rate was low but significantly higher compared to the result obtained in run 47, 2016 (see Table 2).

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

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

## Conclusion
The rmAb clones YR145 and EP10 were the most successful antibodies for immunohistochemical demonstration of CD117. Using one of these two Abs, either in a laboratory develop (LD) assay or in a Ready-to-use (RTU) format, the overall pass rate was 94% and provided a high proportion of optimal results (73%). Efficient HIER, preferable in an alkaline buffer, careful calibration of the primary Ab and use of a 3-step polymer/multimer detection system, provided the highest proportion of optimal result. Optimal results could also be obtained with the pAb A4502, pAb RB-9038-P 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 (58%), but the pass rate was low (52%) and only 6% were assessed as optimal. A similar pattern was

seen with the RTU system based on the rmAb 9.7 (Ventana), where 53% were assessed as sufficient but only 3% were scored as optimal. Both antibodies are difficult to calibrate to an acceptable level and accordingly, less robust for demonstration of CD117.

Appendix is recommended as positive and negative tissue control for CD117. The Cajal cells must show a strong predominantly membranous staining reaction, while smooth muscle cells in muscularis propria and smooth muscle cells surrounding vascular structures must be negative. Mast cells display a strong staining intensity, but cannot be used as reliable control (both internal and external) for monitoring the correct sensitivity level of the assay, as these cells are too easy to stain due to high expression 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 result.

Table 1. **Antibodies and assessment marks for CD117, run 51**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| pAb **A4502** | 160 | Dako/Agilent | 9 | 74 | 68 | 9 | 52% | 59% |
| pAb **RB-9038-P** | 2 | Thermo S./Neomarkers | 1 | 0 | 0 | 1 | - | - |
| pAb **RP063-05** | 1 | ZytoMed Systems | 0 | 0 | 1 | 0 | - | - |
| pAb **E1440** | 1 | Spring Bioscience | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **YR145** | 28<br>6<br>4<br>2<br>1<br>1 | Cell Marque<br>Nordic Biosite<br>Immunologic<br>Epitomics<br>VWR<br>US Biological | 36 | 4 | 2 | 0 | 95% | 94% |
| rmAb clone **EP10** | 5<br>5<br>2<br>1<br>1 | Epitomics<br>Biocare medical<br>Leica/Novocastra<br>Diagnostic Biosystem<br>ZytoMed Systems | 6 | 8 | 0 | 0 | 100% | 100% |
| mAb clone **T595** | 1 | Leica/Novocastra | 0 | 0 | 0 | 1 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| rmAb clone **9.7 790-2951** | 30 | Ventana/Roche | 1 | 15 | 10 | 4 | 53% | 100% |
| rmAb clone **YR145 117R-10-ASR** | 8 | Cell Marque | 5 | 2 | 1 | 0 | 88% | 100% |
| rmAb clone **YR145 KIT-0029** | 1 | Maixin | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **YR145 AN465-5M** | 1 | Biogenex | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **YR145 44218** | 1 | Menarini Diagnostics | 1 | 0 | 0 | 0 | - | - |
| rmAb clone **EP10 PA0007** | 9 | Leica Biosystems | 8 | 1 | 0 | 0 | 100% | 100% |
| rmAb clone **EP10 MAD-000644QD** | 2 | Master Diagnostica | 0 | 2 | 0 | 0 | - | - |
| rmAb clone **EP10 PME-296** | 1 | Biocare Medical | 0 | 0 | 1 | 0 | - | - |
| rmAb clone **EP10 AN818-5M** | 1 | BioGenex | 0 | 0 | 0 | 1 | - | - |
| pAb **PDR045** | 1 | Diagnostic Biosystems | 0 | 0 | 1 | 0 | - | - |
| **Unknown 8267-C010** | 1 | Sakura Finetek USA | 1 | 0 | 0 | 0 | - | - |
| Total | 277 | | 70 | 106 | 84 | 17 | - | |
| Proportion | | | 25% | 38% | 30% | 7% | 63% | |

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 51**
The following protocol parameters were central to obtain optimal staining:

**Concentrated antibodies**
pAb **A4502**: Protocols with optimal results were based on HIER using Cell Conditioning 1 (CC1, Ventana) (4/67) *, Target Retrieval Solution high pH 9 (TRS, 3-in-1, Dako/Agilent) (2/34), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (2/15) or TRIS-EDTA/EGTA pH 9 (1/5) as retrieval buffer. The pAb was typically diluted in the range of 1:75-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 55 of 94 (59%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer).

pAb **RB-9038-P**: One protocol with an optimal result was based on HIER using TRIS-EDTA/EGTA, the pAb was diluted 1:400 and GTVision (Gene Tech, GK6007) was used as the detection system.

rmAb clone **YR145**: Protocols with optimal results were based on HIER using CC1 (Ventana) (24/27), TRS high pH 9 (3-in-1, Dako/Agilent) (4/5), TRS low pH (Dako/Agilent) (1/1), BERS2 (Leica) (2/4), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/1) or Tris-EDTA/EGTA pH 9 (4/4) 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, 34 of 36 (94%) laboratories produced a sufficient staining.

rmAb clone **EP10:** Protocols with optimal results were based on HIER using CC1 (Ventana) (2/5), TRS high pH 9 (3-in-1, Dako/Agilent) (3/3) or BERS2 (Leica) (1/2) as the retrieval buffer. The rmAb was diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 10 of 10 (100%) laboratories produced a sufficient staining.

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 / Omnis | | 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** | 2/41 ** (5%) | 0/1 | 3/53 (6%) | 0/1 | 1/11 (9%) | - |
| rmAb clone **YR145** | 4/5 (80%) | - | 21/23 (91%) | - | 2/3 | 1/1 |
| rmAb clone **EP10** | 3/3 | - | 2/3 | - | 1/3 | - |

* 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, 9 of 9 (100%) laboratories produced a sufficient staining.

rmAb clone **YR145,** product no. **117R-10-ASR,** Cell Marque, Benchmark GX/XT/Ultra:
Protocols with optimal results were based on HIER using CC1, Ventana (efficient heating time 32-72 min. at 95-100°C) and 24-32 min. incubation of the primary Ab. UltraView (Ventana, 760-500) with amplification or OptiView (Ventana, 760-700) with or without amplification was used as detection systems. Using these protocol settings, 7 of 7 (100%) laboratories produced a sufficient staining.

rmAb clone **9.7,** product no. **790-2951,** Roche/Ventana, Benchmark GX/XT/Ultra:
One protocol with an optimal result 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. Using these protocol settings, one laboratory produced a sufficient staining result (optimal).

Table 4 summarises 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 accordingly to the vendor recommendations) and as laboratory modified systems (changing basal protocol settings). Only protocols performed on the specific 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% (3/3) | 100% (3/3) | 100% (6/6) | 83% (5/6) |
| VMS Ultra/XT rm 9.7 pAb **790-4408** | 0% (0/1) | 0% (0/1) | 52% (15/29) | 3% (1/29) |

* 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 and tissue structures expected to be demonstrated. This pattern was observed in 67% of the insufficient results (68 of 101). Too weak staining result in combination with either background and/or false positive reaction was seen in 20% of the insufficient results (20 of 101). 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) and neoplastic cells in the desmoid tumour.

Virtually all laboratories were able to demonstrate CD117 in high-level antigen expressing structures such as neoplastic cells of the GIST, tissue core no. 3, 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. 4, and Cajal cells in the appendiceal muscularis propria was more challenging and required a carefully calibrated protocol.

80% (221 of 277) of the laboratories used a concentrated Ab format within laboratory developed (LD) assays for the demonstration of CD117. The pAb A4502 was the most widely used antibody for the demonstration of CD117. Used as a concentrate by a LD assay, pAb A4502 gave an overall pass rate of 52% (83 of 160). As shown in Table 3, optimal results could be obtained on all three main IHC platforms from Agilent/Dako, Leica and Ventana/Roche, although the proportion of optimal scores was very low (<10%). Protocols providing optimal results were all based on HIER in an alkaline buffer e.g. BERS2 (Leica), CC1 (Ventana) or TRS pH 9 (Dako). However, there was no significant difference in the overall pass rate compared to HIER in an acidic buffer, dilution factor of the primary Ab or 2-step versus 3-step multimer/polymer detection systems. Five protocols omitted HIER altogether, and all were assessed as insufficient (borderline or poor).

As described in previous runs for CD117, the pAb A4509 could provide false positive staining reaction of neoplastic cells in the desmoid tumour. No single parameter could be identified to unravel this aberrant staining pattern, but is most likely related to lot-to-lot-variations of the pAb A4502 (Agilent/Dako). In particular, the lot numbers 10107664, 10118885, 10120930 and 10124187 showed a tendency towards problems related to false positive staining of tissue structures expected to be negative. From a technical point of view, an LD-assay based on the pAb4502 seems difficult to calibrate so it "fit for the purpose" and therefore, the use of the newly introduced rabbit monoclonal antibodies could be a better alternative for detection of CD117 (see below).

In this run, the overall performance of the two rmAbs clone YR145 and EP10 was superior to the pAb A4502 within LD assays. Compared to run 47, the number of laboratories using these two clones has increased significantly, and grouped together, the overall pass rate was 96% (54 of 56). The proportion of optimal results was 75% (42 of 56) and significant higher compared to 6% (9 of 160) obtainable with the pAb A4502 (see Table 1). For both rmAbs (YR145 and EP10), efficient HIER (preferable in a 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 proportion of sufficient results. Using these conditions, and in the dilution range of 1:25-1:1000 of the primary Abs, 100% (31 of 31) of the protocols were assessed as sufficient and 90% (28 of 31) were giving an optimal score.

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). Only one laboratory (3%, 1 of 30) were able to produce an optimal result - see protocol settings above. Noteworthy, nearly all laboratories (29 of 30) used modified protocol settings (see Table 4), and despite using protocol settings producing optimal results with e.g. rmAb YR145 (efficient HIER in CC1 for 32-64 min. and a sensitive detection system as OptiView with or

without amplification), the RTU format 790-2951 (Ventana) is technically challenging and difficult to optimize. The same observation has also been noted in previous runs for CD117.

The RTU format from Cell Marque (117R-10-ASR) based on the rmAb YR145 provided both high proportion of sufficient and optimal results on Benchmark Ultra/XT platforms (Ventana/Roche). Using HIER in CC1 (efficient HIER time for 32-72 min. at 95-100˚C), incubation time of the primary Ab for 16-32 min. 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% (7 of 7) of the protocols were assessed as sufficient and 71% (5 of 7) were giving an optimal mark. The one protocol assessed as insufficient used similar protocol settings except for the use of UltraView Universal Alkaline Phosphatase Red Detection Kit (760-501, Ventana), providing too low sensitivity.

The RTU system from Leica, PA0007 based on the rmAb EP10 was in this run the most effective assay for detection of CD117, providing a pass rate of 100% (9 of 9) of which 89% (8 of 9) of the protocols were assessed as optimal. Both vendor and laboratory modified protocol setting could be used to obtain optimal results (see Table 4).

This was the sixth assessment of CD117 in NordiQC (see Table 2). A pass rate of 63% was obtained, which is an increase compared to the former Run 47, 2016 but still a significant decrease compared to 81% in run 26, 2009. In this run, the performance of pAb A4502 (Dako/Agilent) and the RTU system based on the rmAb 9.7 (Ventana/Roche) was less successful. The overall pass rate for assays based on these two primary Abs was 52% (99 of 190) and only 5% (10 of 190) of the protocols were assessed as optimal. In comparison, the pass rate for all assays (all vendors) using the rmAbs YR145 and EP10 was 94% (75 of 80) and 73% (58 of 80) produced optimal results. These data indicate, that assays based on the rmAbs YR145 or EP10 are more likely to work in a routine setting compared to assays based to the pAb A4502 (Dako/agilent) or the RTU system based on the rmAb 9.7 (Ventana/Roche).
Thus, the use of new highly sensitive and improved rmAbs (YR145 and EP10), setting new standards for optimal performance of a CD117 staining and challenging assays based "older antibodies" used by the majority of participants (e.g. pAb A4502), accounts for the overall low pass rate.

**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.
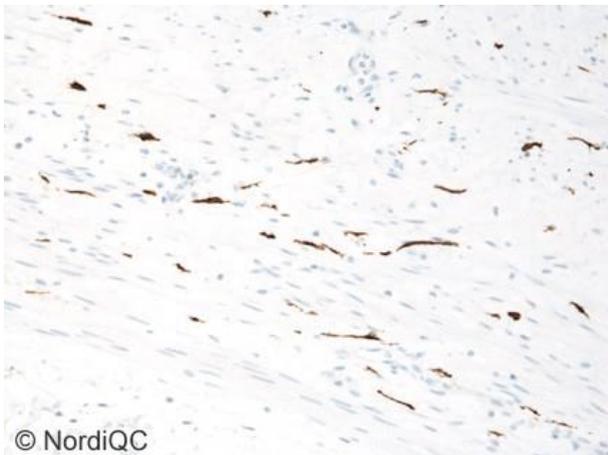


Fig. 1a (x200)
Optimal staining for CD117 of the appendix using the rmAb YR145 as a concentrate, HIER in an alkaline buffer (CC1) and a multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 6a. The Cajal cells in the appendiceal muscularis propria are distinctively stained with strong intensity. The smooth muscle cells are unstained - compare with Fig. 1b.
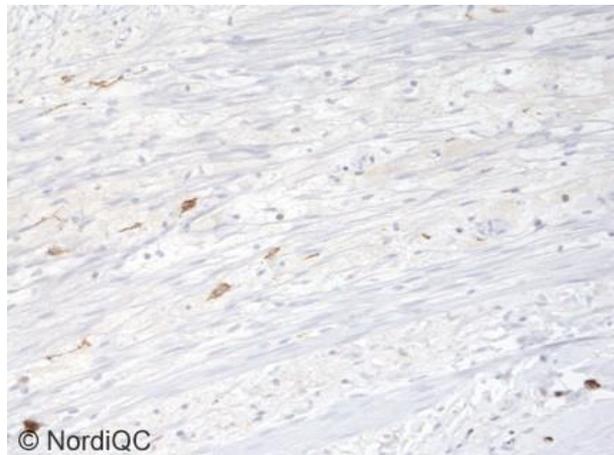
Fig. 1b (x200)
Insufficient staining for CD117 of the appendix using the rmAb clone YR145 with exactly same protocol settings as protocol in Fig 1a., except for the use of the less sensitive multimer based detection system Ultraview (Ventana) – same protocol used in Figs. 2b – 6b. The proportion of Cajal cells are significantly reduced displaying a weak staining intensity - compare with Fig. 1a (same field).
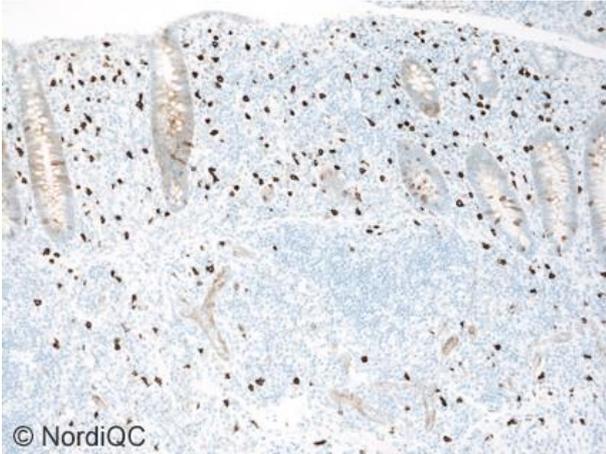
Fig. 2a (x100)
Optimal staining for CD117 in the appendix using same
protocol as in Fig. 1a. The mast cells show a strong
staining reaction, neovascular structures and the basal
compartment of the epithelium show a weak to
moderate staining intensity.
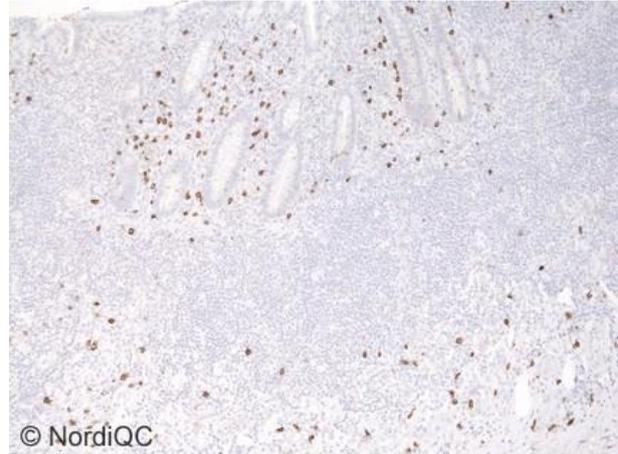


Fig. 2b (x100)
Insufficient staining for CD117 in the appendix using
same protocol as in Fig. 1b. Mast cells are stained with
reduced intensity but the neovascular structures and
epithelial cells in the crypts are completely negative -
compare with Fig. 2a (same field).
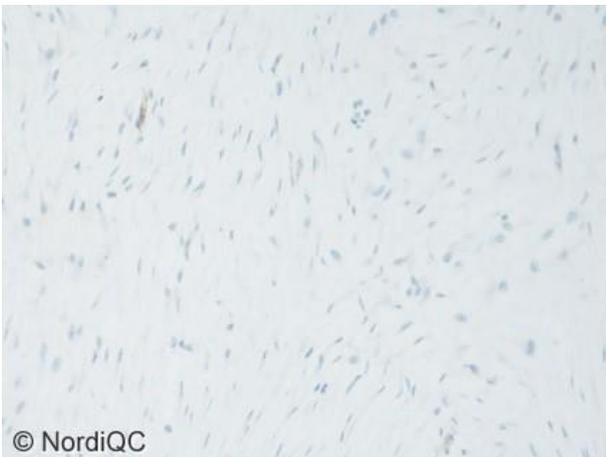


Fig. 3a (x200)
Optimal staining for CD117 in the desmoid tumour using
same protocol as in Fig. 1a. All the neoplastic cells are
negative and only a faint staining reaction can be seen
in endothelium in scattered neovascular structures.
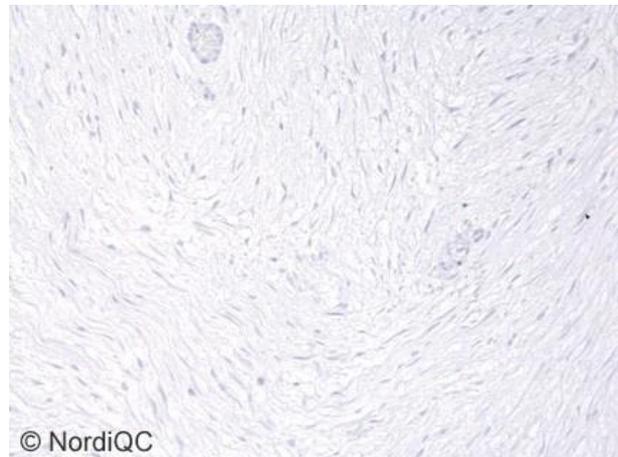


Fig. 3b (x200)
Insufficient staining for CD117 in the desmoid tumour
using same protocol as in Fig. 1b. Although the reaction
pattern is as expected, the protocol provides low
sensitivity - compare with Fig. 3a (same field) but also
with Fig. 1a-1b, 2a-2b, 5a-5b and 6a-6b.
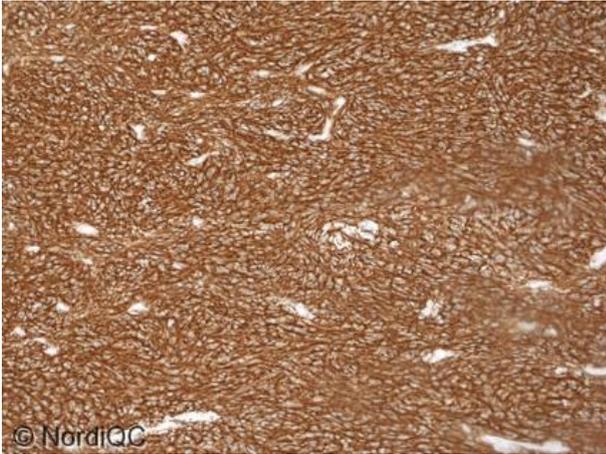
Fig. 4a (x200)
Optimal staining for CD117 of the GIST, core no 3, using same protocol as in Fig. 1a - 3a. All the neoplastic cells are strongly stained.
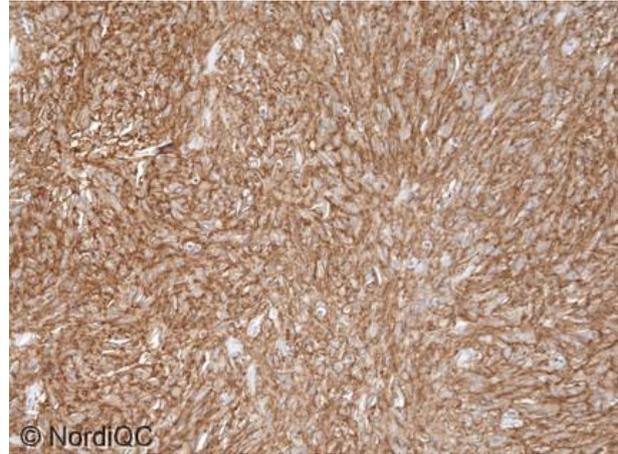

Fig. 4b (x200)
Staining for CD117 of the GIST, core no 3, using same protocol as in Fig. 1b -3b. Staining intensity of the neoplastic cells is as expected - compare with Fig. 4a (same field). However, also compare with Fig. 1a-1b, 2a-2b, 5a-5b and 6a-6b.
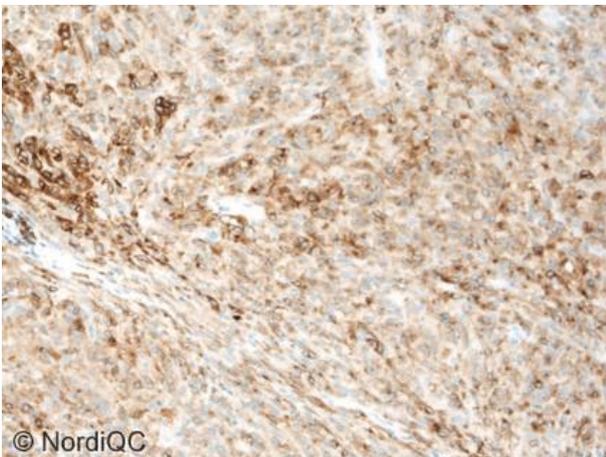

Fig. 5a (x200)
Optimal staining for CD117 of the GIST, core no 4, using same protocol as in Fig. 1a - 4a. Virtually all the neoplastic cells show a weak to moderate but distinct staining reaction.
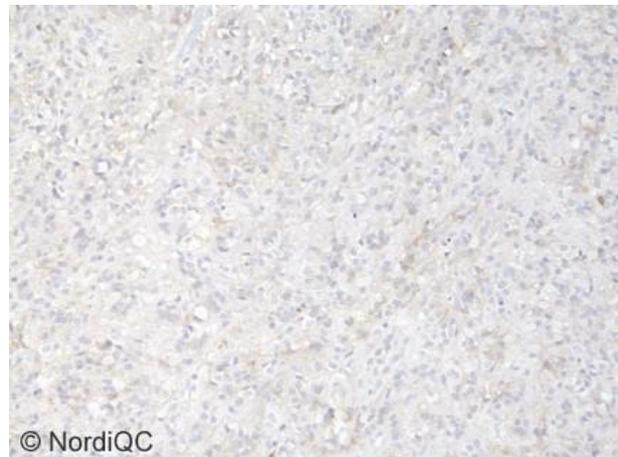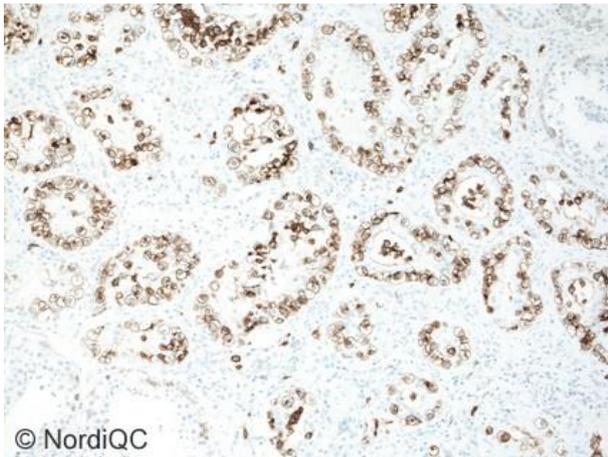

Fig. 5b (x200)
Insufficient staining for CD117 of the of the GIST, core no 4, using same protocol as in Fig. 1b - 4b. The vast majority of neoplastic cells are completely negative or only faintly stained - compare with Fig. 5a (same field).

Fig. 6a (x100)
Optimal staining for CD117 of the GCNIS using same protocol as in Fig. 1a - 5a. All intratubular neoplastic cells in the testis displays a strong, distinct membranous staining reaction.
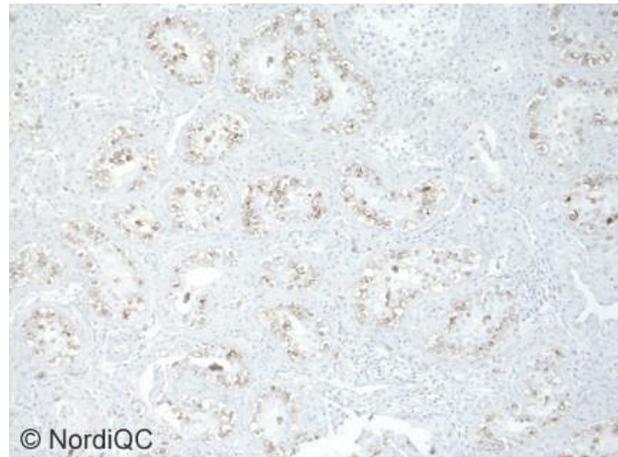


Fig. 6b (x100)
Insufficient staining for CD117 of the GCNIS using same protocol as in Fig. 1b - 5b. The proportion of stained intratubular neoplastic cells in the testis is reduced and intensity is too weak - compare with Fig. 6a (same field).
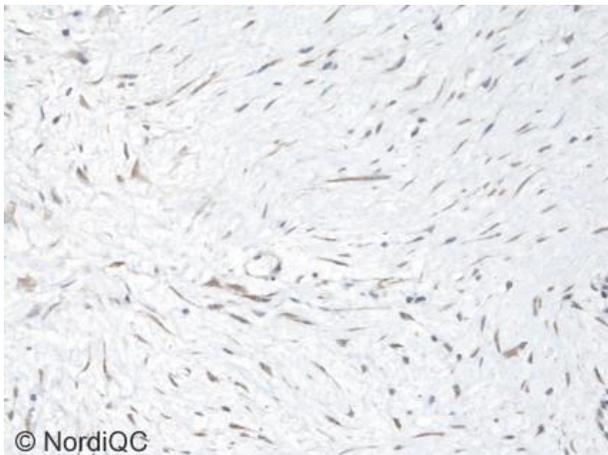


Fig. 7a (x200)
False positive staining for CD117 of the desmoid tumour using the pAb A4502 (lot no. 10107664, Dako/Agilent) as concentrate, and with same protocol settings as in Fig. 1a - 6a. The neoplastic cells show a distinct but aberrant cytoplasmic staining reaction - compare with optimal result in Fig. 3a.
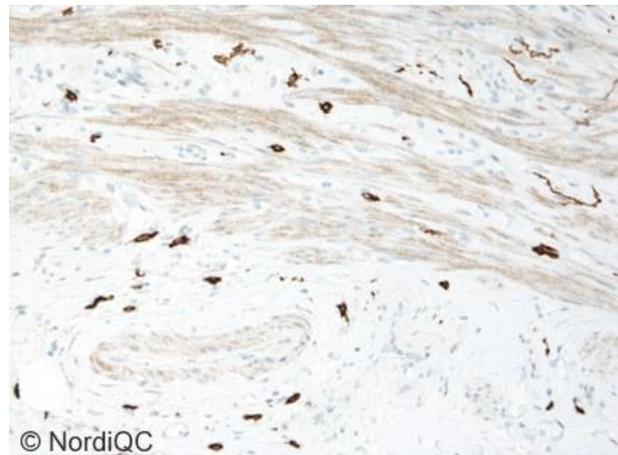


Fig. 7b (X200)
False positive staining for CD117 of the appendix using the pAb A4502 as concentrate (1:250), HIER in CC1 combined with the use of OptiView 760-700 (Ventana/Roche) as the detection system. The Cajal cells display the expected level of expression, whereas the smooth muscle cells (appendiceal lamina muscularis proria and arterial walls) are aberrantly labelled compromising interpretation. The antibody pAb A4502 within a LD-assay is difficult to calibrate precisely although participants use, as shown in this example, optimal protocol settings.

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