Assessment Run 46 2016
CD34

Material
The slide to be stained for CD34 comprised:
1. Appendix, 2. Liver, 3. Leiomyoma, 4. Pre-B-ALL, 5. Gastrointestinal stromal tumour (GIST), 6. Dermatofibrosarcoma protuberans
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD34 staining as optimal included:

- A strong and distinct, predominantly membranous staining reaction of virtually all endothelial cells in all tissues included.
- A moderate to strong, predominantly membranous staining reaction of Cajal cells in muscularis propria and stromal fibroblast-like cells in the appendix.
- A moderate to strong, predominantly membranous staining reaction of the endothelial cells in the portal tracts and of the periportal sinusoidal endothelial cells in the liver (the zone 1 sinusoids).
- A strong, distinct membranous staining reaction of virtually all neoplastic cells in the Pre-B-ALL.
- A strong, predominantly membranous staining reaction of virtually all neoplastic cells of the gastrointestinal stromal tumour and of the dermatofibrosarcoma protuberans.
- No staining reaction of the epithelial cells in the appendix and in the liver. Smooth muscle cells in all tissue cores including benign smooth muscle cells in the leiomyoma should be negative.

Participation

Number of laboratories registered for CD34, run 46	298
Number of laboratories returning slides	278 (93%)

Results
277 laboratories participated in this assessment and one laboratory submitted an inappropriate primary antibody. 265 (95%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Omission of HIER
- Unexplained technical issues

Performance history
This was the third NordiQC assessment of CD34. The overall pass rate was high and improved compared to the result obtained in run 30, 2010 (see table 2).

Table 2. **Proportion of sufficient results for CD34 in the three NordiQC runs performed**

	Run 7 2003	**Run 30 2010**	**Run 46 2016**
Participants, n=	64	169	277
Sufficient results	75%	86%	95%

Conclusion
The mAb clones **QBEnd 10** and **BS72** could be used to obtain optimal staining results for CD34. The mAb QBEnd 10 is a very robust antibody and used by 99% of the participants either within a laboratory developed (LD) assay or as a ready-to-use (RTU) format. No significant difference in the performance was observed between these two entities with an overall pass rate of 95%. Efficient HIER, the use of appropriate titre of the primary Ab and incubation time tailored to the choice of IHC system were the most important prerequisites for an optimal staining result. The Ready-To-Use system of mAb clone QBEnd 10 (PA0212), Leica provided the highest proportion of optimal results. Liver is recommended as positive and negative tissue controls: The endothelial cells of the portal tracts and the periportal sinusoidal endothelial cells must show a moderate to strong predominately membranous staining reaction. No reaction must be seen in hepatocytes.

Table 1. **Antibodies and assessment marks for CD34, run 46**

Concentrated antibodies:	N	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **QBEnd 10**	70 28 7 5 5 4 2 2 1 1 1 1 1 1	Dako/Agilent Leica/Novocastra Immunologic Thermo S./NeoMarkers Cell Marque Becman C./Immunotech Biocare Bio SB AbD Serotec Spring Bioscience GeneMed A.Menarini Diagnostics Vector Nordic Biosite	74	49	5	1	95%	96%
rmAb clone **EP88**	3	BD Biosciences	0	2	1	0	-	-
mAb clone **BS72**	1	Nordic Biosite	1	0	0	0	-	-
Ready-To-Use antibodies:								
mAb clone **QBEnd 10, 790-2927**	76	Ventana/Roche	49	25	2	0	97%	98%
mAb clone **QBEnd 10, IR632**	39	Dako/Agilent	28	8	2	1	92%	96%
mAb clone **QBEnd 10, GA632**	15	Dako	10	5	0	0	100%	100%
mAb clone **QBEnd 10, PA0212**	9	Leica/Novocastra	9	0	0	0	100%	100%
mAb clone **QBEnd 10, MAD-01613QD**	2	Master Diagnostica	1	1	0	0		
mAb clone **QBEnd 10, PM 084**	1	Biocare	1	0	0	0	-	-
mAb clone **QBEnd 10, BP003**	1	ID-Labs	1	0	0	0	-	-
mAb clone **QBEnd 10, KIT-0004**	1	Maixin	1	0	0	0	-	-
Total	277		175	90	10	2	-	
Proportion			63%	32%	4%	1%	95%	

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

Detailed analysis of CD34, Run 46
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **QBEnd 10**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using either Target Retrieval Solution pH 9 (Dako) (8/9)*, Target Retrieval Solution pH 9 (3-in-1) (Dako) (13/19), Target Retrieval Solution pH 6.1 (3-in-1) (Dako) (4/5), Cell Conditioning 1 (Ventana) (26/57), Bond Epitope Retrieval Solution 2 (Leica) (11/13), Diva Decloaker pH 6.2 (BioCare) (1/2), EDTA/EGTA pH 8 (2/2), Tris-EGTA/EDTA pH 9 (5/8) , Citrate pH 6 (2/4) or Citrate pH 6.7 (1/1) as retrieval buffer. One protocol with an optimal result was based on a combination of proteolytic pre-treatment in Protease 1 (Ventana) and HIER in Cell Conditioning 1 (Ventana) (1/1). The mAb was typically diluted in the range of 1:20-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 95 of 99 (96%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **BS72**: One protocol with an optimal result was based on HIER using Tris-EGTA/EDTA pH 9 (efficient heating time 20 min. at 98°C), 30 min. incubation of the primary Ab, a titre of 1:150 and BioSiteHisto Plus HRP Polymer anti-Mouse kit (KDB-10007, Nordic Biosite) as detection system.

Table 3. **Proportion of optimal results for CD34 for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Omnis / Autostainer (Link)**		**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
mmAb **QBEnd 10**	16/21** (76%)	3/3	23/48 (48%)	-	9/10 (90%)	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
mAb clone **QBEnd 10**, product no. **790-2927**, Ventana, BenchMark XT/Ultra:
Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (efficient heating time of 8-64 min at 95-100°C), 12-32 min. incubation at 36-37°C of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 61 of 62 (98%) laboratories produced a sufficient staining.
By omission of HIER, 16-32 min. incubation at 37°C of the primary Ab and UltraView with amplification (760-500+760-080) or OptiView (760-700) as detection system, three laboratories were able to produce an optimal staining result.

mAb clone **QBEnd 10**, product no. **IR632**, Dako, Autostainer Link:
Protocols with optimal results were based on HIER using TRS pH 9 or TRS pH 6 (efficient heating time for 10-30 min. at 95-98°C), 12-30 min. incubation at room temperature in the primary Ab and EnVision FLEX/FLEX+(K8000/K8002) as detection system. Using these protocol settings 25 of 26 (96%) laboratories produced a sufficient staining (optimal or good).

mAb clone **QBEnd 10**, product no. **GA632**, Dako, Omnis:
Protocols with optimal results were based on HIER using TRS pH 9 (efficient heating time for 30 min. at 97°C), 12.5 min. incubation of the primary Ab at 32°C and EnVision FLEX+ (GV800/GV821) as detection system. Using these protocol settings 11 of 11 (100%) laboratories produced a sufficient staining (optimal or good).

mAb clone **QBEnd 10,** product no. **PA0212**, Leica/Novocastra, BOND III/MAX:
Protocols with optimal results were based on HIER using BERS1 or BERS2 (efficient heating time for 5-30 min. at 97-100°C), 15-30 min. incubation of the primary Ab at room temperature and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings 9 of 9 (100%) produced an optimal staining.

mAb clone **QBEnd 10,** product no. **MAD-01613QD**, Master Diagnostica:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (efficient heating time for 20 min. at 97°C), 20 min. incubation of the primary Ab at room temperature and MAD-000237QK/N as detection system.

mAb clone **QBEnd 10,** product no. **PM 084**, Biocare:
One protocol with an optimal result was based on HIER using Diva Decloaker pH 6.2 (efficient heating time for 15 min. at 110°C), 30 min. incubation of the primary Ab at room temperature and MACH4 Universal HRP-Polymer (M4U534) as detection system.

mAb clone **QBEnd 10,** product no. **KIT-0004**, Maixin:
One protocol with an optimal result was based on HIER in a pressure cooker using Citrate buffer pH 6 (efficient heating time 2 min. at 120°C), 60 min. incubation of the primary Ab at room temperature and Maixin, KIT-5230 as detection system.

Comments
In this third NordiQC run for CD34, a pass rate of 95% was observed. Only 12 of 277 (5%) laboratories obtained an insufficient mark due to a too weak staining intensity or false negative reaction in cellular structures expected to be demonstrated. Virtually all participating laboratories were able to demonstrate CD34 in the GIST and in the dermatofibrosarcoma protuberans with high level CD34 expression. Demonstration of CD34 in endothelial cells of small vessels e.g. located in the appendiceal lamina propria, the neoplastic cells of the Pre-B-ALL and the endothelial cells of the periportal sinusoids in the liver was more challenging and could only be demonstrated with appropriate protocol settings.

The mAb clone QBEnd 10 was used by 99% (273 of 277) of the participating laboratories. Optimal results could be obtained both as a concentrated format within a laboratory developed (LD) assay and as a

Ready-To-Use (RTU) format. As a concentrate, mAb clone QBEnd 10 provided optimal staining results on all three main IHC systems, see table 3. No special requirements for choice of HIER buffer and detection system were needed to provide optimal staining results as long as careful calibration of the primary Ab titer was performed and adjusted to the overall sensitivity of the IHC system. For example, the mAb clone QBEnd 10, prod.no. M7165, Dako provided an optimal result both by a protocol based on HIER for 30 min. in TRS low pH pH 6.1 (Dako), a titre of 1:20 and a highly sensitive 3-step polymer based detection system (FLEX+, GV800/821, Dako) and also by HIER for 30 min. in TRS pH 9, a titre of 1:100 of the primary Ab and a less sensitive 2-step polymer based system (FLEX, GV800, Dako). In general, this demonstrates that the mAb clone QBEnd 10 is a robust antibody, and optimal results can be produced both by HIER in alkaline and non-alkaline buffers, 2- and 3-step detection systems providing the titre of the primary Ab is adjusted.

In this assessment, 52% (144 of 277) of the laboratories used Ready-To-Use (RTU) antibodies. There was no significant difference in the overall performance compared to laboratory developed (LD) assays (see table 1). All RTU formats were based on the mAb clone QBEnd 10 and sufficient and optimal results could be obtained with all RTU systems from the respective vendors, both by the use of vendor recommended protocol settings and by laboratory modified protocols.
Best performance was obtained with the RTU system, PA0212 from Leica as all 9 protocols based on this product gave an optimal result.
A very high proportion of sufficient and optimal results was also seen with the RTU formats from Dako (IR632/GA632) and Ventana (790-2927). For the Ventana RTU system, laboratory modified protocol settings applying HIER as pre-treatment provided a significantly higher proportion of optimal results compared to the level seen if the official recommended settings omitting pre-treatment were applied. By omission of HIER and using the RTU format 790-2927 with UltraView as detection kit a pass rate of 86% was seen, but none were assessed as optimal. However, if OptiView was used as detection system 3 of 3 protocols gave an optimal result. When HIER was performed in CC1 (30-36 min. at 95-100°C) and the RTU format was used together with either UltraView or OptiView, a pass rate of 100% (23 of 23) was seen, of which 74% (17 of 23) were optimal. All nine protocols (100%) based on OptiView were assessed as optimal and 57% (8 of 14) of the protocols based on UltraView gave an optimal score. In this context it is advisable to use a sensitive detection system but more important, to perform HIER in order to overcome antigen masking problems related to the fixation in formalin and thus improving the staining precision leading to better staining results and enhancing optimal performance as shown above.

This was the third assessment of CD34 in NordiQC (see table 2). A pass rate of 95% was obtained, which is a significant improvement compared to 86% in run 30, 2010. Any concrete details to the specific causes for the weak or false negative staining in the remaining 5% of the protocols is difficult to elucidate as most protocol settings were similar to protocols otherwise given optimal results. In run 30, 10% (17 of 169) of the protocols were based on omission of HIER, and in this run only 5% (13 of 277) of the participating laboratories were using the same condition in their protocol set-up. Although the mAb clone QBEnd 10 is a very robust antibody and can provide optimal results even without pretreatment, it is recommendable to use HIER either in acidic or alkaline buffers (preferable) and carefully calibrate the titre of the primary antibody tailored to the detection system of choice.

Controls
Liver and appendix is recommended as positive and negative tissue controls for CD34. In liver the protocol must be calibrated to provide a moderate to strong predominately membranous staining reaction of endothelial cells in the portal vessels, but also of the periportal sinusoidal endothelial cells serving as "low expressors" for an optimal calibrated assay for CD34. No staining reaction must be seen in liver cells.
As a supplement to liver, appendix can be used. All endothelial cells and, in particular endothelial cells lining the small vessels in lamina propria, Cajal cells in muscularis propria and stromal fibroblast-like cells must be stained as strongly as possible without any staining reaction of smooth muscle cells and epithelial cells.

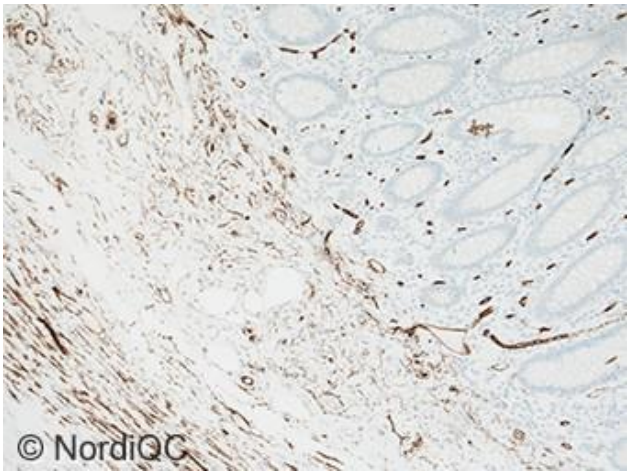

Fig. 1a (x100)
Optimal CD34 staining of the appendix using the mAb clone QBEnd 10 as a concentrate by a laboratory developed (LD) assay, HIER in an alkaline buffer (CC1) and a multimer based detection system (OptiView, Ventana) - same protocol used in Figs. 2a - 5a.
The endothelial cells of the small vessels in the lamina propria, Cajal cells in the muscularis propria and fibroblastic-like cells especially located in the submucosa/muscularis propria are strongly stained, while the epithelial cells are negative - compare with Fig. 1b.

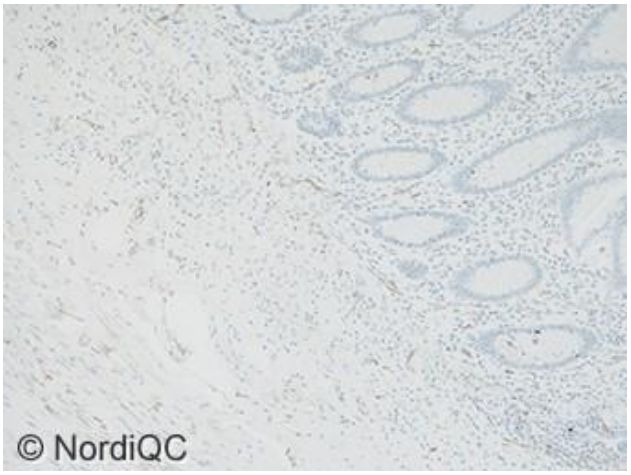

Fig. 1b (x100)
Insufficient CD34 staining of the appendix using the mAb clone QBEnd 10 as a concentrate by a LD assay, HIER in an alkaline buffer (CC1) and a multimer based detection system (Ultraview, Ventana) - same protocol used in Figs. 2b-5b.
The proportion and intensity of cells stained is significantly reduced compared to the level expected and most likely due to a too low concentration of the primary Ab not being calibrated correctly to the chosen less sensitive detection system as used in Fig. 1a (same field).

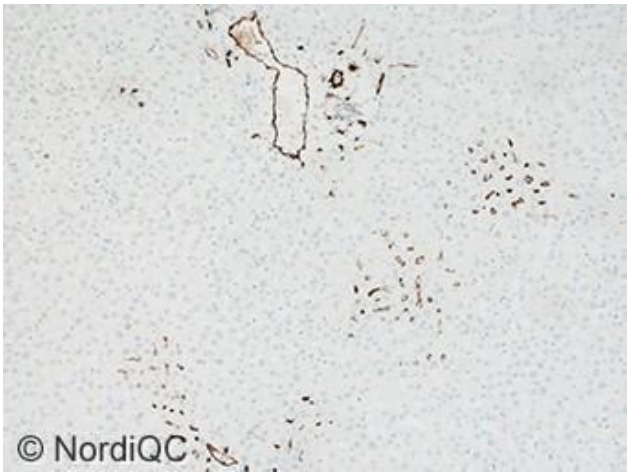

Fig. 2a (x100)
Optimal CD34 staining of the liver using same protocol as in Fig. 1a.
The endothelial cells of the portal tracts and of the periportal sinusoids show a moderate to strong, predominately membranous staining reaction. Liver cells are negative - compare with Fig. 2b.

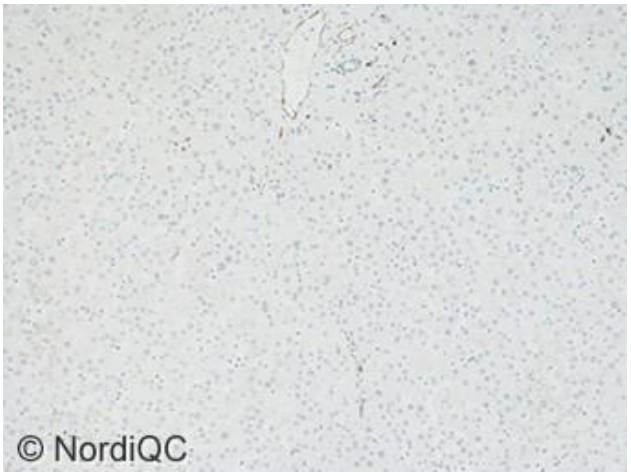

Fig. 2b (x100)
Insufficient CD34 staining of the liver using same protocol as in Fig. 1b.
The endothelial cells of the portal tracts and of the periportal sinusoids are false negative or only show a weak/equivocal staining reaction - compare with Fig. 2a

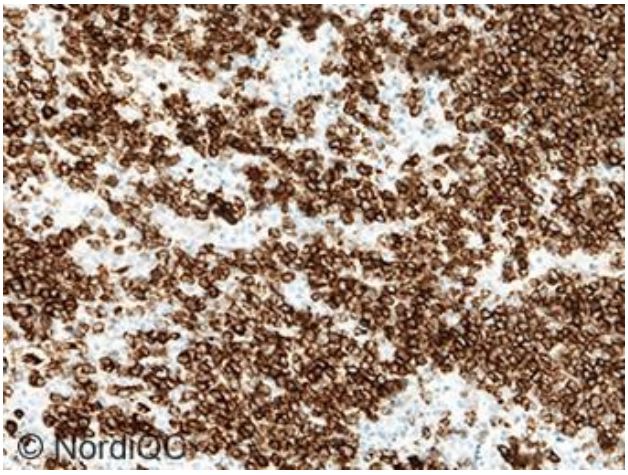

Fig. 3a (x200)
Optimal CD34 staining of the Pre-B-ALL using same protocol as in Fig. 1a & 2a.
Virtually all neoplastic B-cells show a strong, predominately membranous staining reaction - compare with Fig. 3b.

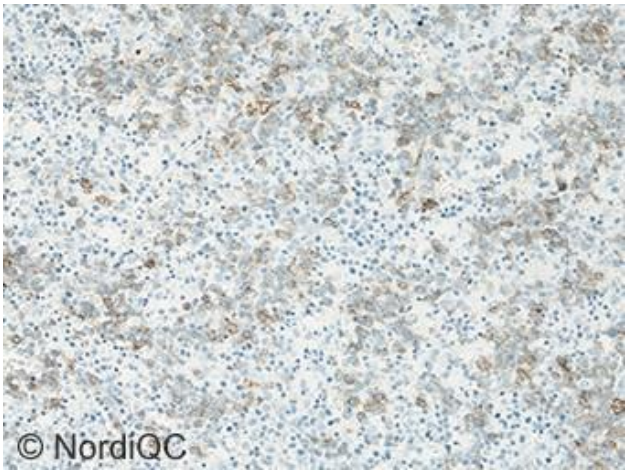

Fig. 3b (x200)
Insufficient CD34 staining of the Pre-B-ALL using same protocol as in Fig. 1b & 2b.
The majority of neoplastic B-cells show a weak/ equivocal staining reaction or are completely false negative - compare with Fig. 3a (same field).

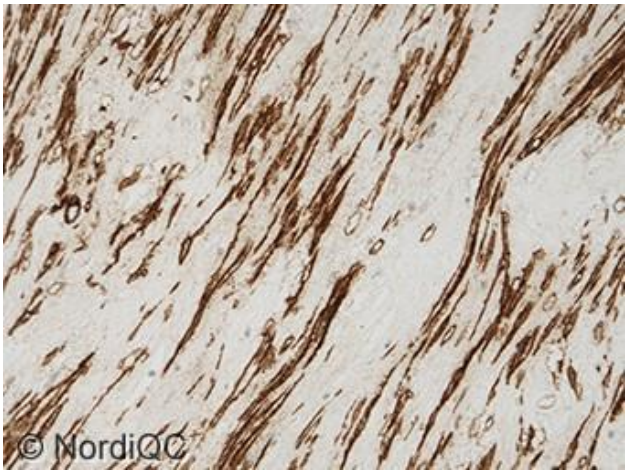

Fig. 4a (x200)
Optimal CD34 staining of the GIST using same protocol as in Fig. 1a-3a.
All the neoplastic cells show a strong, predominately membranous staining reaction - compare with Fig. 4b.

Fig. 4b (x200)
Insufficient CD34 staining of the GIST using same protocol as in Fig. 1b-3b.
Although the majority of the neoplastic cells are stained, the staining intensity is significantly reduced and only faint membranous staining reaction can be identified - compare with Fig. 4a (same field).

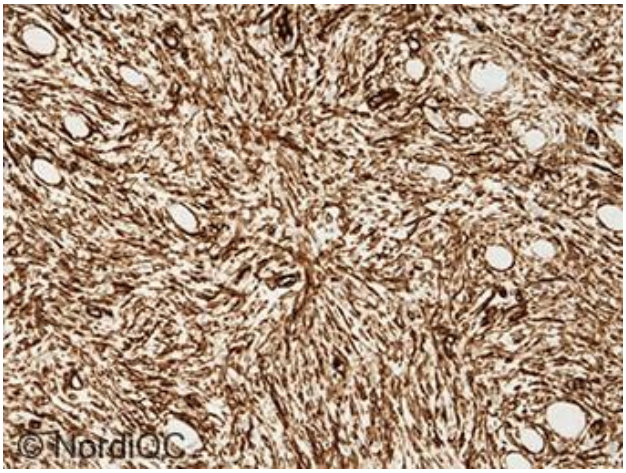

Fig. 5a (x200)
Optimal CD34 staining of the dermatofibrosarcoma protuberans using same protocol as in Fig. 1a-4a.
All neoplastic cells show a strong, predominately membranous staining reaction - compare with Fig. 5b.

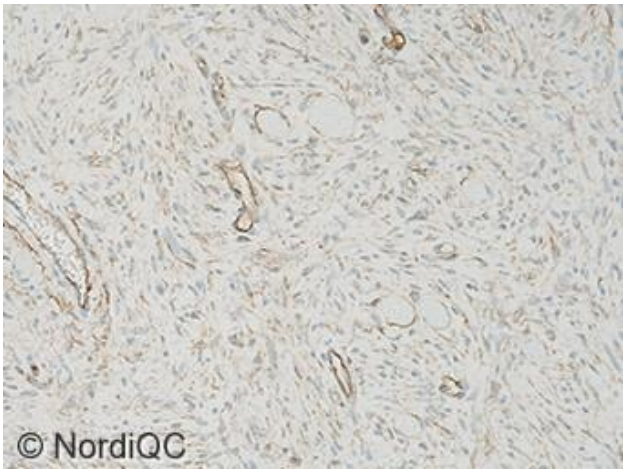

Fig. 5b (x200)
Insufficient CD34 staining of the dermatofibrosarcoma protuberans using same protocol as in Fig. 1b-4b.
The neoplastic cells are stained, but the intensity and proportion of cells demonstrated is significantly reduced and barely visible - compare with Fig. 5a (same field).

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