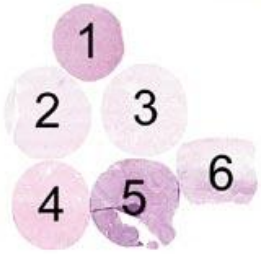
Assessment Run 30 2010
CD34

The slide to be stained for CD34 comprised:



1. Spleen with acute lymphoblastic leukaemia (Pre-B-ALL), 2. Gastrointestinal stromal tumour, 3. Leiomyoma, 4. Liver, 5. Tonsil, 6. Appendix

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD34 staining as optimal included:

- A moderate to strong cytoplasmic reaction with membrane accentuation of virtually all the endothelial cells in all the tissues included.
- A moderate to strong cytoplasmic reaction with membrane accentuation of the Cajal cells in the appendiceal muscularis propria.
- A moderate to strong, distinct cytoplasmic reaction with membrane accentuation of the sinusoidal endothelial cells in the vicinity of the portal tracts in the liver (the zone 1 sinusoids).
- A strong, distinct membranous reaction of virtually all the neoplastic cells of the Pre-B-ALL and the gastrointestinal stromal tumour.
- No staining reaction in the neoplastic cells of the leiomyoma and the epithelium of the appendix.

169 laboratories participated in this assessment. 86 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks given are summarized.

Table 1. **Abs and assessment marks for CD34, run 30**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **QBEnd10**	53 23 22 4 2 1 1 1 1 1 1 1 1	Dako Leica/Novocastra NeoMarkers Beckman C./Immunotech Serotec Biocare Cell Marque Cymbus Biotechnology Euro-diagnostica Master Diagnostica Monosan Sigma Vector Zytomed Systems	63	32	16	2	84 %	91 %
mAb clone **My10**	4	BD Biosciences	1	2	0	1	-	-
mAb clone **EP373Y**	1	Epitomics	0	1	0	0	-	-
pAb **8G12**	1	BD Biosciences	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone **QBEnd 10, 790-2927**	26	Ventana	16	8	2	0	92 %	100 %
mAb clone **QBEnd 10, IR632**	15	Dako	7	8	0	0	100 %	100 %
mAb clone **QBEnd 10, PA0212**	3	Leica/Novocastra	3	0	0	0	-	-
mAb clone **QBEnd 10, PM 084**	1	Biocare	1	0	0	0	-	-
mAb clone **QBEnd 10, IS632**	1	Dako	1	0	0	0	-	-
mAb clone **QBEnd 10, BP003**	1	ID-Labs	1	0	0	0	-	-

mAb clone **QBEnd 10, MS-363-R7**	1	NeoMarkers	1	0	0	0	-	-
mAb clone **QBEnd 10, 134M-18**	1	Cell Marque	0	1	0	0	-	-
mAb clone **QBEnd 10, PN IM1185**	1	Beckman C./Immunotech	0	0	1	0	-	-
Total	169		94	52	20	3	-	-
Proportion			56 %	30 %	12 %	2 %	86 %	-

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

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **QBEnd 10**: Except for one lab (out of 6) who performed no pre-treatment, the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Tris-EDTA/EGTA pH 9 (18/25)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (14/25), Cell Conditioning 1 (BenchMark, Ventana) (11/25), Bond Epitope Retrieval Solution 2 (Bond, Leica) (7/10), Citrate pH 6 (6/10), Diva Decloaker (Biocare) (2/2), Bond Epitope Retrieval Solution 1 (Bond, Leica) (2/3) or EDTA/EGTA pH 8 (2/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:150 depending on the total sensitivity of the protocol employed. Using these protocol settings 59 out of 65 (91 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **My10**: The protocol giving an optimal result was based HIER using Target Retrieval Solution pH 9 (3-in-1) (Dako). The mAb was diluted 1:20 and visualized with EnVision FLEX+ (prod.no. K8002, Dako).

Ready-To-Use Abs
mAb clone **QBEnd 10** (prod. no. 790-2927, Ventana): The protocols giving an optimal result were primarily based on HIER, but an optimal result could also be obtained by omission of retrieval. Using HIER with Cell Conditioning 1 (BenchMark, Ventana) 12 out of 16 protocols resulted in an optimal result, whereas the remaining 4 protocols were assessed as good. 1 laboratory used HIER in EDTA/EGTA pH 8.
By omission of HIER, 3 out of 7 protocols gave an optimal result, whereas 3 were assessed as good.
The primary Ab was incubated in the range of 12 - 32 min. and both UltraView (760-500 or 760-501) and Iview (760-091) could be used as the detection kit.

mAb clone **QBEnd 10** (prod. no. IR632, Dako): The protocols giving an optimal result were all based on HIER using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH) (7/7), an incubation time of 20-30 min in the primary Ab and EnVision Flex/Flex+(K8000/K8002) as the detection system. Using these protocol settings 12 out of 12 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **QBEnd 10** (prod. no. PA0212, Leica/Novocastra): The protocols giving an optimal result were all based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica) (3/3), an incubation time of 15 min. in the primary Ab and Bond Polymer Refine (DS9800) as the detection system. Using these protocol settings 3 out of 3 (100%) produced an optimal staining.

mAb clone **QBEnd 10** (prod. no. PM 084, Biocare): The protocol giving an optimal result was based on HIER using Borg Decloaker pH 9.5 (Biocare), an incubation time of 30 min. in the primary Ab and MACH 3 Polymer (M3M530, Biocare) as the detection system.

mAb clone **QBEnd 10** (prod. no. IS632, Dako): The protocol giving an optimal result were based on HIER using Tris-EDTA/EGTA pH 9, an incubation time of 30 min. in the primary Ab and EnVision (K5007, Dako) as the detection system.

mAb clone **QBEnd 10** (prod. no. BP003, ID-Labs): The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica), an incubation time of 24 min. in the primary Ab and Bond Polymer Refine (DS9800) as the detection system.

mAb clone **QBEnd 10** (prod. no. MS-363-R7, Thermo S./ Neomarkers): The protocol giving an optimal result was based on HIER using Cell Conditioning 1 (Ventana), an incubation time of 32 min in the primary Ab and iView (760-091, Ventana) as the detection system.

The most frequent causes of insufficient stains were:
- Too low concentration of the primary antibody
- Inappropriate epitope retrieval - e.g., all of 3 protocols based on enzymatic pre-treatment for the mAb clone QBEnd 10 gave an insufficient result
- Omission of HIER

In this assessment and in concordance to the previous assessment of CD34, run 7 2003, the prevalent feature of an insufficient staining was a too weak or false negative staining. Virtually all laboratories were able to demonstrate CD34 in high antigen expressing structures such as the endothelial cells in large vessels and in the neoplastic cells of the gastrointestinal stromal tumour. Staining of endothelial cells of the small vessels, the Cajal cells in the appendiceal muscularis propria, the neoplastic cells of the Pre-B-ALL and the endothelium of the periportal sinusoids in the liver was more challenging due to a lower expression of CD34 and thus required an optimally calibrated protocol. The mAb QBEnd 10 was the most commonly used Ab for detection of CD34 and sufficient results could be obtained by HIER or by omission of pre-treatment. However, the pass rate for laboratories omitting pre-treatment was only 71% (10/14) compared to a pass rate of 89 % for laboratories performing HIER (136/152). Even though omission of HIER could be used to obtain an optimal staining, the staining intensity, staining precision and the proportion of positive cells in e.g., the endothelial cells of small vessels in lamina propria in the appendix and in the tonsil was slightly reduced compared to the results obtained by HIER. From a general perspective HIER is recommended to eliminate or reduce the influence of the fixation time in formalin.
All protocols based on enzymatic pre-treatment were assessed as insufficient (3 out of 3) due to an excessive digestion of the tissue causing loss of the membranes and cytoplasm and consequently giving a false negative staining. In this context it is noteworthy that vendors like Dako and Leica/Novocastra still recommend proteolytic pre-treatment when using the mAb clone QBEnd10 as a concentrate (whereas HIER is recommended when the clone is sold as a Ready-To-Use (RTU) format from same vendors!).
An aberrant false positive nuclear staining was seen in various cell types in stains from 4 laboratories using the mAb clone QBEnd 10 in an RTU format, Ventana, combined with HIER in Cell Conditioning 1 and UltraView (760-500) as the detection system on the BenchMark XT, Ventana. These were assessed as good. No explanation for this aberrant staining pattern could be identified (as e.g. the same lot number and similar protocol settings also could give an optimal result without nuclear staining).

This was the second assessment of CD34 in NordiQC. The proportion of sufficient results increased from 75 % in run 7, 2003 to 86 % in the current run – see table 2. This result is encouraging, taken into consideration the many new participants. A pass rate of 92 - 100 % for the most widely used Ready-To-Use formats/systems for CD34 from Ventana and Dako may have contributed to the increase and to the overall very satisfactory performance.

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

	Run 7 2003	Run 30 2010
Participants, n=	64	169
Sufficient results	75 %	86 %

As control, liver displayed the most informative reaction pattern as a critical stain quality indicator for CD34. In the optimal protocols, the endothelial cells of the periportal sinusoids and portal tracts showed a distinct and moderate to strong predominantly membranous staining reaction. In the insufficient staining deemed too weak, the endothelial cells of the periportal sinusoids were negative or displayed only a weak/equivocal reaction pattern.
Alternatively, appendix can be used as control, in which the Cajal cells in the muscularis propria and virtually all the endothelial cells of the small vessels in lamina propria must show a moderate to strong staining, while the epithelium must be negative.

Conclusion
The mAb clones QBEnd 10 and My10 are both recommendable Abs for CD34. The mAb clone QBEnd 10 was in this assessment a robust marker both as a concentrate and as Ready-To-Use format. For all clones HIER should be used for an optimal performance. Liver is an appropriate control for CD34: The endothelial cells surrounding the portal tracts must show a moderate to strong distinct predominantly membranous staining, while the liver cells must be negative.

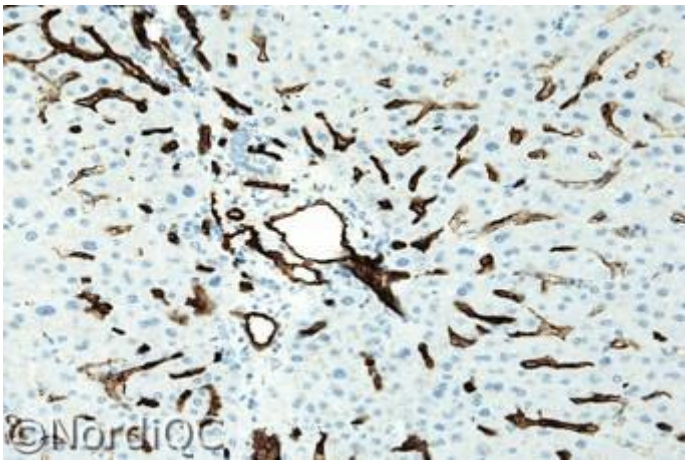


Fig. 1a
Optimal CD34 staining of the liver using the mAb clone QBEnd 10 carefully calibrated after HIER in an alkaline buffer. The endothelial cells of both the portal vessels and the periportal sinusoids show a moderate to strong distinct staining (x200).

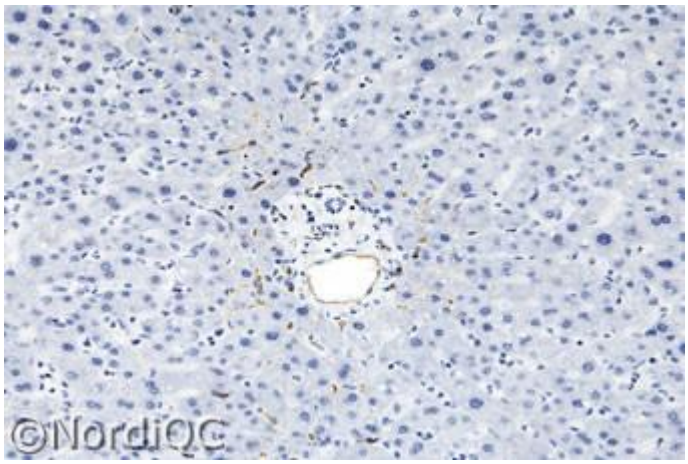


Fig. 1b
Insufficient CD34 staining of the liver using the mAb clone QBEnd 10 too diluted - same field as in Fig. 1a.
The intensity and proportion of the staining in the endothelial cells of both the portal vessels and in particular the periportal sinusoids is significantly reduced compared to the expected level seen in Fig. 1a (x200).

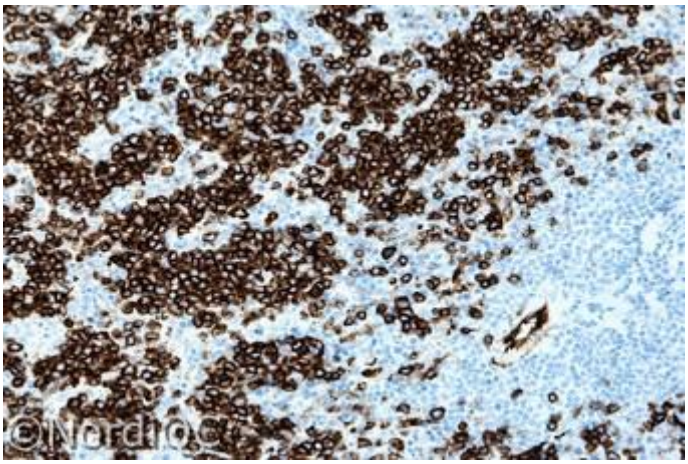


Fig. 2a
Optimal CD34 staining of the Pre-B-ALL using the mAb clone QBEnd 10 with the same protocol as in Fig. 1a.
A strong, predominantly membranous staining is seen in virtually all the neoplastic cells (x200).

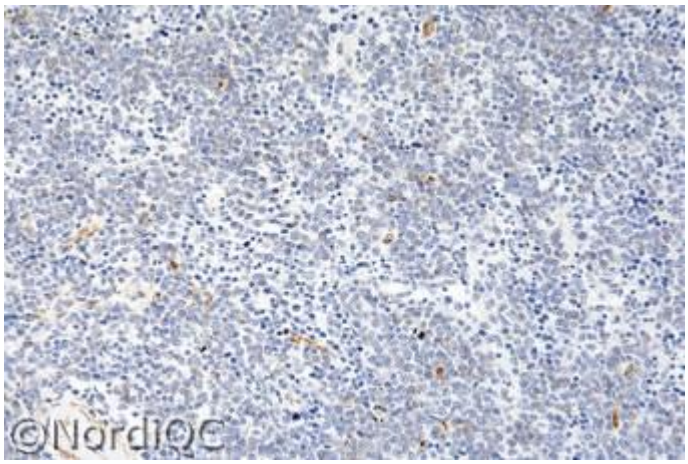


Fig. 2b
Insufficient CD34 staining of the Pre-B-ALL using the mAb clone QBEnd 10 with the same protocol as in Fig. 1b. Only the endothelial cells are demonstrated, while the neoplastic cells of the B-ALL are negative (x200).

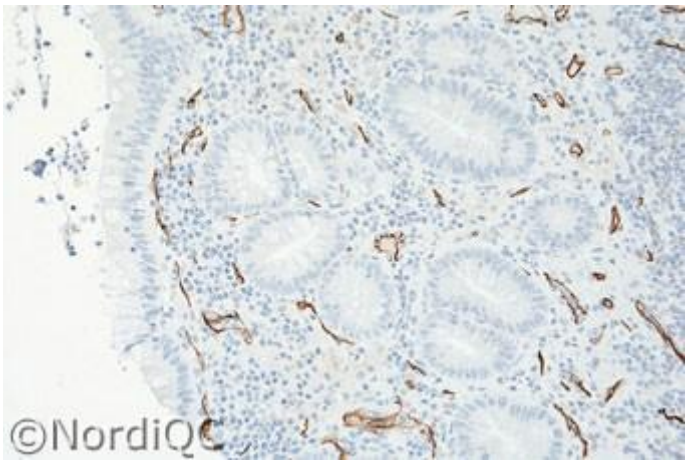


Fig. 3a
Optimal CD34 staining of the appendix using the mAb clone QBEnd 10 as Ready-To-Use, Ventana with HIER in CC1. The endothelial cells in the small vessels beneath the epithelium show a strong and crisp staining (x200).

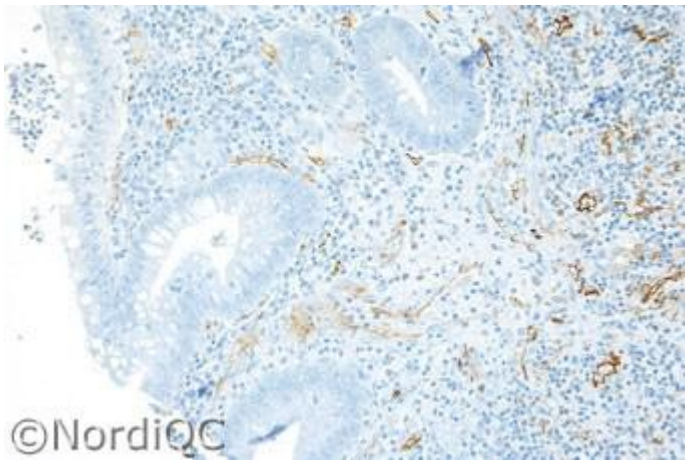


Fig. 3b
CD34 staining of the appendix using the mAb clone QBEnd 10 as Ready-To-Use, Ventana without HIER. The endothelial cells in small vessels beneath the epithelium show a reduced and less crisp staining compared to the level seen in Fig. 3a. (x200) The other tissues in the multitissue block stained as expected.

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