Assessment Run 44 2015

DOG1 (Discovered on GIST-1)

Material
The slide to be stained for DOG1 comprised:

1. Tonsil, 2. Appendix, 3. Leiomyosarcoma, 4-5. Gastrointestinal stromal tumour (GIST)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing DOG1 staining as optimal included:

- An at least moderate, distinct predominantly membranous staining reaction of the vast majority of Cajal cells in the appendix
- A weak membranous staining reaction of scattered endothelial cells and vascular smooth muscle cells in all included tissues
- A weak membranous staining reaction of the majority of columnar epithelial cells in the basal part of the crypts of the appendix
- A moderate to strong staining reaction of virtually all neoplastic cells in the two GISTs
- No staining reaction of neoplastic cells of the leiomyosarcoma

For the rmAb clone SP31, staining of lymphocytes was accepted. At present no conclusive data on the background of this reaction pattern are available and consequently staining reaction of lymphocytes was excluded from the analysis and scoring.

Participation

Number of laboratories registered for DOG1, run 44	167
Number of laboratories returning slides	153 (92%)

Results
153 laboratories participated in this assessment. Of these, 109 (71%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Use of less sensitive detection systems
- Too low concentration of the primary antibody
- Insufficient HIER (too short efficient heating time)

Performance history
This was the first NordiQC assessment of DOG1. A pass rate of 71% was observed.

Table 2. **Proportion of sufficient results for DOG1 in the first NordiQC run performed**

	Run 44 2015
Participants, n=	153
Sufficient results	71%

Conclusion
The mAb clone **K9** and the rmAb clone **SP31** could both be used to obtain optimal staining results for DOG1. Irrespective of the clone applied, efficient HIER, use of sensitive (preferably 3-step) detection systems and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. Compared to concentrated formats and laboratory developed assays, the Ready-To-Use systems from Leica and Ventana provided the highest proportion of sufficient and optimal results. Appendix is recommended as positive and negative tissue control: Virtually all Cajal cells must show an at least moderate and distinct predominantly membranous staining reaction, while a weak staining reaction in columnar epithelial cells in the basal crypts must be seen. No staining reaction should be seen in smooth muscle cells of muscularis mucosae and muscularis propria.

Table 1. **Antibodies and assessment marks for DOG1, run 44**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **1.1**	1	Biogenex	0	0	1	0	-	-
mAb clone **DOG1.1**	1 3 1	Biocare Diagnostics Biosystems Zytomed	0	2	2	1	-	-
mAb clone **K9**	64	Leica/Novocastra	15	24	18	7	61%	62%
rmAb clone **SP31**	10 9 5 4 1 1 1	Cell Marque Immunologic Thermo/NeoMarkers Spring Bioscience Genetech Thermo/Pierce Zytomed	11	14	3	3	81%	87%
Unknown	1	Unknown	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **1.1** **MAD-000446QD**	3	Master Diagnostica	1	0	2	0	-	-
mAb clone **1.1** **AM570**	2	Biogenex	0	1	1	0	-	-
mAb clone **DOG1.1** **PM385**	1	Biocare	0	0	1	0	-	-
mAb clone **K9** **PA0219**	8	Leica/Novocastra	3	3	1	1	75%	86%
rmAb **SP31** **760-4590**	29	Ventana/Cell Marque	13	13	2	1	90%	90%
rmAb clone **SP31** **244R-18**	5	Cell Marque	1	4	0	0	100%	-
rmAb clone **SP31** **RMA610/Kit-0035**	3	Maixin	1	2	0	0	-	-
Total	153		46	63	31	13	-	
Proportion			30%	41%	20%	9%	71%	

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

Detailed analysis of DOG1, Run 44

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **K9**: Protocols with optimal results were based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (3/14)*, TRS pH 9 (Dako) (1/10), Cell Conditioning 1 (CC1, Ventana) (3/19), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (6/10) or BERS1 (Leica) (2/4) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:100. Using these protocol settings 29 of 47 (62%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **SP31**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako) (3/5), TRS pH 9 (Dako) (2/4), CC1 (Ventana) (4/10), CC2 (Ventana) (1/2) or Tris-EDTA pH 9 (1/5) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 20 of 23 (87%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for DOG1 for the two most commonly used antibodies as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**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
mAb clone **K9**	5/16** (31%)	0/3	3/16 (19%)	-	6/6 (100%)	2/3
rmAb clone **SP31**	4/7 (57%)	0/1	4/9 (44%)	1/2	-	-

* 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 **K9**, product no. **PA0219**, Leica, Bond Max, Bond-III:
Protocols with optimal results were based on HIER using BERS2 pH 9 or BERS1 pH 6 (Bond, Leica) (efficient heating time 20 min. at 99-100°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine kit (DS9800) as detection system. Using these protocol settings 6 of 7 (86%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **SP31**, product no. **760-4590**, Ventana, BenchMark XT, ULTRA:
Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (efficient heating time 24-64 min.), 8-60 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit or OptiView (760-700) as detection system. Using these protocol settings 26 of 29 (90%) laboratories produced a sufficient staining result.

Comments
In this first NordiQC assessment of DOG1, the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 82% of the insufficient results (36 of 44 laboratories). Too weak staining result was typically characterized by reduced staining reaction both in regard to the intensity and proportion of cells expected to be demonstrated. This was in particular observed in Cajal cells of the appendix and the GIST tissue core no 4, whereas virtually all laboratories successfully demonstrated DOG1 in the majority of neoplastic cells of the GIST tissue core no 5.
The remaining insufficient results were characterized by a general poor signal-to-noise ratio, excessive background reaction and/or aberrant nuclear staining reaction in cells not expressing DOG1 compromising the interpretation.

Optimal staining results could be obtained with the mAb clones K9, 1.1 and the rmAb SP31 (see table 1). It was observed that the rmAb clone SP31 gave an unexpected staining reaction of T-cells in all tissue cores included in this run. It is uncertain whether this staining reaction of T-cells is a true positive reaction, but since this staining pattern did not interfere with the specific reaction in cells expected to be positive for DOG1, it was fully accepted.

The mAb clone K9 was the most widely used antibody as concentrated format within a laboratory developed (LD) assay and provided optimal results on all three main IHC platforms from Dako, Leica and Ventana, respectively (see table 3). mAb clone K9 gave an overall pass rate of 61% (39 of 64) of which 23% were optimal. For optimal performance with mAb clone K9, efficient HIER in an alkaline buffer in combination with a sensitive 3-step polymer or multimer based detection system seem to be the most critical parameters.
If the concentrated format of mAb clone K9 was used in the range of 1:50-100, HIER in ER2 pH 9 and performed on the BOND platform using Refine (3-step polymer) as detection system, all 6 of 6 protocols submitted gave an optimal result.
Concerning HIER, it was observed that not only the choice of buffer but also the efficient HIER time had a major impact on the pass rate and proportion of optimal results. This was e.g. observed for the Dako Autostainer platform using PT-Link as HIER device. If efficient HIER was performed at 95-98°C and < 20 min., only 1 of 6 protocols gave a sufficient result (17%), none of which were optimal. One the other hand, if HIER was performed for 20-25 min. at 95-98°C, 10 of 15 protocols 67% were assessed as sufficient and 27% optimal.

The rmAb clone SP31 applied as a concentrate within a LD assay provided a higher pass rate and proportion of optimal results compared to mAb clone K9. Overall, the same protocol settings, as range of titer, HIER buffer, HIER time and detection systems were applied by the laboratories using the two clones indicating that rmAb clone SP31 might be slightly more robust and easier to calibrate for an optimal result.

Corresponding Ready-To-Use (RTU) systems for mAb clone K9 and rmAb clone SP31 from Leica and Ventana, respectively, provided increased pass rates and proportion of optimal results compared to LD assays.
The Ventana RTU system based on the rmAb clone SP31 was the most successful RTU system giving the highest overall pass rate and proportion of optimal results. Optimal results for the RTU formats were typically obtained by usage of the official protocol recommendations given by the vendors. Laboratory modified protocol settings (typically adjusting HIER, incubation time of the primary Ab and/or choice of detection system) could also provide sufficient and optimal results.

Controls
Appendix can be recommended as positive and negative tissue control for DOG1. A moderate to strong predominantly membranous staining must be seen in virtually all Cajal cells, whereas a weak staining reaction must be seen in basal columnar epithelial cells, scattered endothelial and smooth muscle cells of vessels. No staining reaction should be seen in mast cells and smooth muscle cells of muscularis propria and lamina muscularis mucosae.

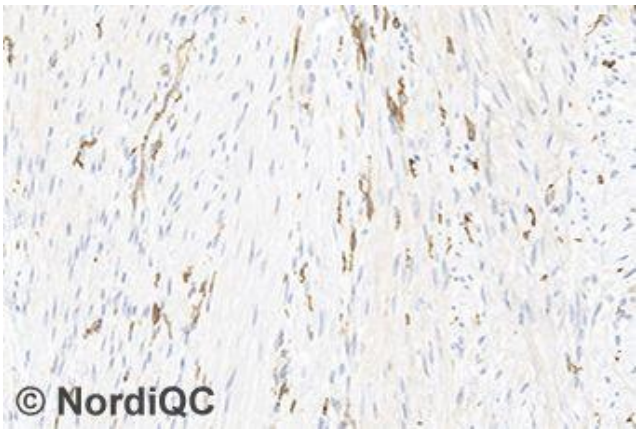

Fig. 1a (x200)
Optimal DOG1 staining of appendix using the mAb clone K9 as a concentrate, optimally calibrated using HIER in an alkaline buffer (CC1 pH 8.5, Ventana) and a 3-step multimer based detection system (OptiView, Ventana). The Cajal cells show a moderate and distinct pre-dominantly membranous staining reaction - compare with Fig.1b. Same protocol used in Figs. 2a - 4a.

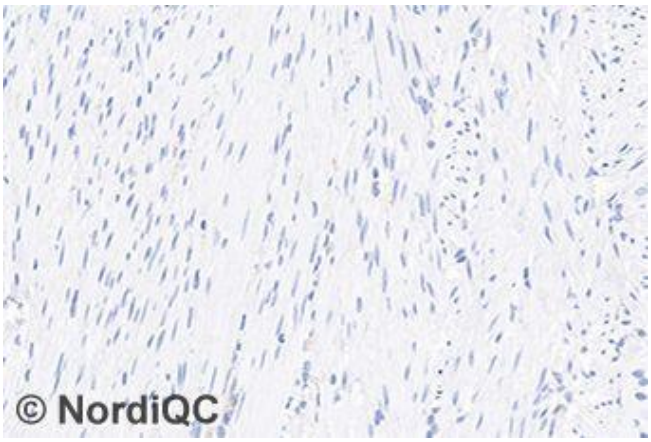

Fig. 1b (x200)
Insufficient DOG1 staining of appendix using the mAb clone K9. The protocol provided an overall too low sensitivity most likely due to a combination of insufficient HIER in Tris-EGTA/EDTA pH 9 (too short time), low sensitivity of the detection system and/or too low concentration of the primary Ab - compare with Fig. 1a (same field). Only few Cajal cells show a weak staining reaction. Also compare with Figs. 2b - 3b – same protocol.

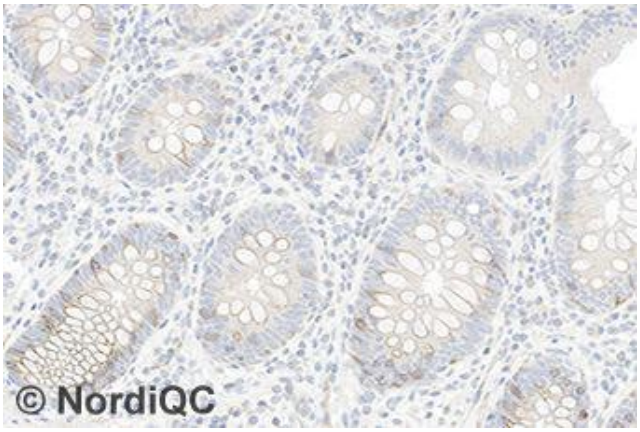

Fig. 2a (x200)
Optimal DOG1 staining of appendix using same protocol as in Fig. 1a. Scattered epithelial show a weak to moderate pre-dominantly membranous staining reaction. No background staining is seen.

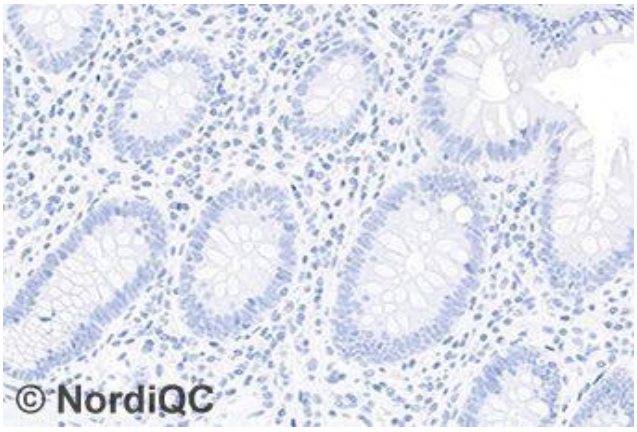

Fig. 2b (x200)
Insufficient DOG1 staining of appendix using same protocol as in Fig. 1b. All epithelial cells are completely false negative - compare with Fig. 2a (same field). Also compare with Figs. 3b – same protocol.

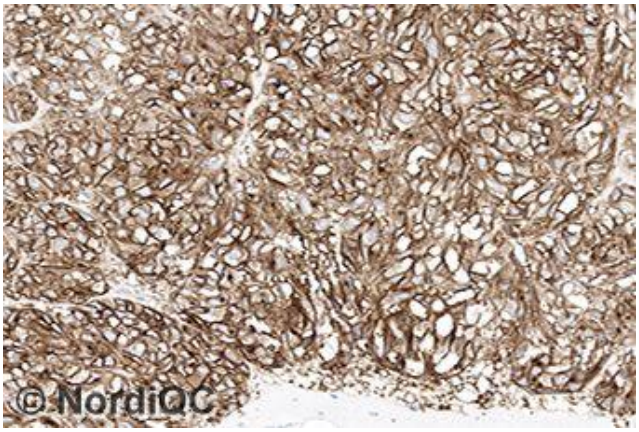

Fig. 3a (x200)
Optimal DOG1 staining of the GIST, tissue core no 4, using same protocol as in Figs. 1a & 2a. Virtually all neoplastic cells show a moderate to strong membranous staining reaction and dot-like positivity.

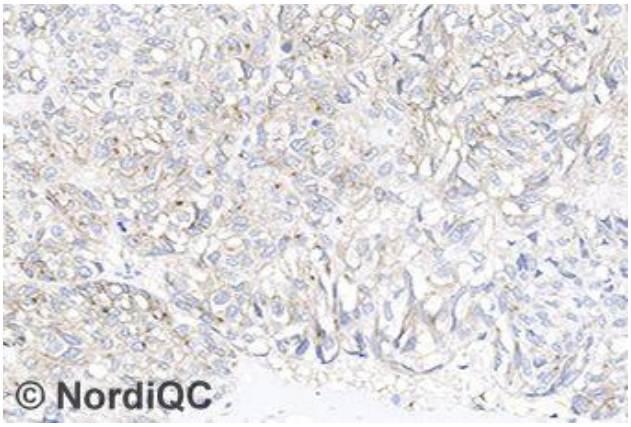

Fig. 3b (x200)
Insufficient DOG1 staining of the GIST, tissue core no 4, using same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak and equivocal staining reaction - compare with Fig. 3a (same field).

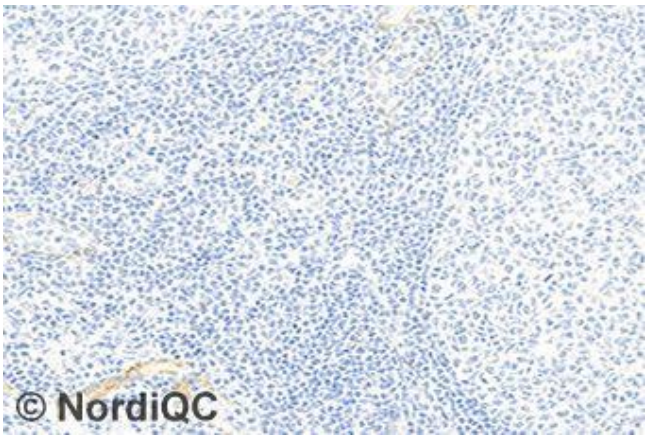

Fig. 4a (x100)
Optimal DOG1 staining of the tonsil using same protocol as in Figs. 1a - 3a. T- and B-cells are negative and only endothelial cells and smooth muscle cells of vessels show a weak staining reaction.

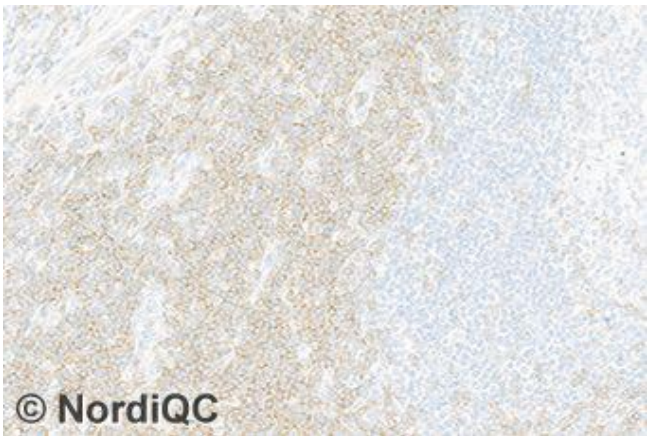

Fig. 4b (x100)
Optimal DOG1 staining of the tonsil using rmAb clone SP31. T-cells show a weak to moderate membranous staining reaction. The staining pattern was accepted if otherwise an optimal staining result was observed. At present no data are available on DOG1 expression in T-cells.

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