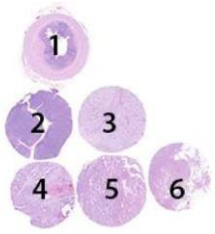
Assessment Run 54 2018

Podoplanin (Podop)

Material
The slide to be stained for Podop comprised:



1. Appendix, 2. Tonsil, 3. Seminoma, 4. Embryonal carcinoma,
5. Lung squamous cell carcinoma, 6. Mesothelioma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Podop staining as optimal included:

- A strong, distinct predominantly cytoplasmic staining reaction of the lymphatic endothelial cells in all tissues.
- A weak to moderate, distinct predominantly cytoplasmic staining reaction of the Schwann cells of the muscularis propria in the appendix.
- A strong, distinct predominantly cytoplasmic staining reaction of the follicular dendritic cells in the germinal centres and the basal squamous epithelial cells in the tonsil, tissue core no. 2.
- A strong, distinct predominantly membranous staining reaction of the neoplastic cells in the seminoma, tissue core no. 3, and mesothelioma, tissue core no. 6.
- A weak to moderate (apical) membranous staining reaction in the majority of the neoplastic cells in the embryonal carcinoma, tissue core no. 4.
- A negative staining reaction of the neoplastic cells of the lung squamous cell carcinoma, tissue core no. 5, and epithelial cells in appendix and tonsil.

Participation

Number of laboratories registered for Podop, run 54	243
Number of laboratories returning slides	225 (93%)

Results
225 laboratories participated in this assessment. 145 (64%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes 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 or too high concentration of the primary Ab
- Insufficient Heat Induced Epitope Retrieval (HIER)
- Low analytical sensitivity of the Ventana/Roche 760-4395 RTU
- False positive staining with the Dako/Agilent IR/IS072 RTU.

Performance history
This was the third NordiQC assessment of Podop. A small increase of the pass rate was seen compared to run 36, 2012.

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

	Run 19 2007	Run 36 2012	Run 54 2018
Participants, n=	29	102	225
Sufficient results	69%	59%	64%

Conclusion
Optimal staining results could be obtained with the mouse monoclonal Ab (mAb) clones **D2-40** and **MX025.** Virtually all laboratories (222 of 225) used mAb clone D2-40. Efficient HIER, preferably in alkaline buffer, use of a sensitive polymer/multimer detection system and careful calibration of the primary Ab were the most important prerequisites for an optimal staining result. Although Podop is a challenging marker, the concentrated format of the mAb clone D2-40 provided optimal staining results on all four main stainer platforms – Dako Autostainer, Dako Omnis, Leica Bond and Ventana BenchMark.
In general, the performance of the Ready-To-Use (RTU) systems was inferior to the laboratory developed (LD) assays using the Abs in concentrated format. The proportion of sufficient staining results for the most widely used RTU-systems (clone D2-40 based), Ventana/Roche 760-4395 and Dako/Agilent IR/IS072 was

only 51% and 42%, respectively. The 760-4395 RTU showed low analytical sensitivity on the Ventana BenchMark platform, whereas the IR/IS072 RTU was troubled by false positive staining reactions on the Dako Autostainer. In contrast, the IR/IS072 performed much better on the Dako Omnis platform. No false positive staining reaction and a general pass rate of 95% was seen, indicating a more efficient washing procedure might be needed for this RTU on the Dako Autostainer.

Appendix is the recommended positive and negative tissue control for Podop. An at least weak to moderate cytoplasmic staining of Schwann cells in the muscularis propria of the appendix must be seen. In addition, endothelial cells of lymphatic vessels should be strongly stained, whereas no staining reaction should be seen in endothelial cells of the blood vessels and in the columnar epithelial cells and goblet cells of the mucosa.

Table 1. **Antibodies and assessment marks for Podop, run 54**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **18H5**	1	Acris Antibodies	0	1	0	0	-	-
mAb clone **4D5aE5E6**	1	ThermoFisher	0	0	0	1	-	-
mAb clone **D2-40**	66 8 4 3 3 2 2 1 1 1	Dako/Agilent Cell Marque Biocare Covance Zytomed Systems Acris Antibodies Signet BioLegend Diagnostic BioSystems ThermoFisher	42	22	14	13	70%	78%
Ready-To-Use antibodies								
mAb clone **D2-40 PM266AA**	1	BioCare	0	1	0	0	**-**	**-**
mAb clone **D2-40 322M**	9	Cell Marque	3	4	1	1	-	-
mAb clone **D2-40 IS/IR072**	26	Dako/Agilent	5	6	11	4	42%	42%
mAb clone **D2-40 IR/IS072** [3]	19	Dako/Agilent	11	7	1	0	95%	-
mAb clone **D2-40 IR/IS072** [4]	7	Dako/Agilent	4	2	0	1	-	-
mAb clone **D2-40 MAD-000402QD**	2	Master Diagnostica	1	1	0	0	-	-
mAb clone **D2-40 8515**	1	Sakura Finetek	1	0	0	0	**-**	**-**
mAb clone **D2-40 760-4395** [5]	65	Ventana/Roche	14	19	13	19	51%	77%
mAb clone **D2-40 760-4395** [6]	1	Ventana/Roche	0	0	0	1	-	-
mAb clone **MX025 MAB-0714**	1	Maixin	1	0	0	0	-	-
Total	225		82	63	40	40	-	
Proportion			36%	28%	18%	18%	64%	

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer) but used by laboratories on the full-automatic Dako Omnis system.
4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer) but used by laboratories on different platforms (e.g. Ventana Benchmark and Leica Bond).
5) Best results with laboratory modified protocols, see Table 4.
6) RTU system developed for the Ventana BenchMark system but used by laboratories on different platforms (e.g. Dako Autostainer)

Detailed analysis of Podop, Run 54
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **D2-40**: Protocols with optimal results were all based on heat induced epitope retrieval (HIER) using Cell Conditioning Solution 1 (CC1, Ventana/Roche) (22/50)*, Bond Epitope Retrieval Solution 2 (BERS2, Leica) (8/11), Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9 (3-in-1), Dako/Agilent) (6/8), TRS High pH (Dako/Agilent) (3/6), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/5), Tris-EDTA/EGTA pH 9 (1/2) or Montage EDTA Antigen Retrieval Solution pH 8 (Diagnostic BioSystems) (1/1). The mAb was diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 56 of 71 (78%) laboratories produced an optimal staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

Table 3. **Proportion of optimal results for Podop for the most commonly used antibodies as concentrates on the 4 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
mAb clone **D2-40**	5/6** (83%)	0/1	3/6 (50%)	-	20/42 (48%)	0/1	8/10 (80%)	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
mAb clone **D2-40**, product no. **IR072 or IS072**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 95-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX (K8000/K8002) (2-step polymer system) as detection system. Using these protocol settings, 8 of 19 (42%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **D2-40**, product no. **MAD-000402QD**, Master Diagnostica, MD-Stainer:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 for 20 min., 10 min. incubation of the primary Ab and Master Polymer Plus (MAD-000230QP) as detection system.

mAb clone **D2-40,** product no. **8515**, Sakura Finetek, Genie:
One protocol with an optimal result was based on 30 min. HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer, 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system.

mAb clone **D2-40**, product no. **760-4395**, Ventana/Roche, Ventana Benchmark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time for 32-72 min. at 100°C), 32-60 min. incubation at 36°C of the primary Ab and 3-step multimer detection systems, UltraView (760-500) with amplification (760-080), OptiView (760-700) or OptiView with tyramide signal amplification (760-099 / 860-099). Using these protocol settings, 23 of 30 (77%) laboratories produced sufficient staining results.

mAb clone **MX025**, product no. **MAB-0714**, Maixin, manual:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (Water bath) for 20 min., 60 min. incubation of the primary Ab and MaxVision III DAB as detection system.

Table 4 summarizes 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 by 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 Podop for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb clone **D2-40, IR072 or IS072**	43% (6/14)	14% (2/14)	42% (5/12)	25% (3/12)
VMS mAb clone **D2-40, 760-4395**	14% (2/14)	0% (0/14)	61% (31/51)	27% (14/51)

* 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 integrated.

Comments

In this assessment and in concordance with the previous NordiQC assessments of Podop, the prevalent features of an insufficient staining were either a generally too weak or false negative staining reaction of the cells and structures expected to be demonstrated (see Figs. 7a – 9b) or a false positive staining reaction (see Figs. 1a – 4b). Too weak or false negative staining reaction was seen in 78% of the insufficient results (62 of 80 laboratories). False positive staining was seen in 18 laboratories (22%). Virtually all laboratories were able to demonstrate Podop in the neoplastic cells of the seminoma and the mesothelioma and in the majority of the lymphatic endothelial cells in all tissue cores, whereas the demonstration of Podop in the follicular dendritic cells in the germinal centres of the tonsil and in particular the Schwann cells of the appendix and the neoplastic cells of the embryonal carcinoma were more challenging and required an optimally calibrated protocol. False positive staining reaction was seen in 18 cases. Typically, false positive staining reaction was seen in the majority of goblet cells in the appendix – alone or in combination with false positive staining reaction in a proportion of the neoplastic cells in the lung squamous carcinoma.

41% (93 of 225) of the laboratories used Abs in a concentrated format within LD-assays for Podop (see Table 1). Virtually all laboratories used mAb clone D2-40 (91 of 93) and optimal staining results could only be obtained with this clone. Optimal staining results could be obtained on all major stainer platforms (Dako Autostainer, Dako Omnis, Ventana BenchMark and Leica Bond) – see Table 3. Irrespective of the stainer platform used, careful calibration of the titre and efficient HIER at high pH were the main protocol prerequisites for optimal results. Both 2- and 3-step polymer/multimer based detection systems could be used to provide an optimal result, but the vast majority of laboratories (93%) used a 3-step polymer/multimer based detection system to get optimal results. For the LD-assays, the prevalent features of an insufficient staining were a generally too weak or false negative staining reaction of the cells and structures expected to be demonstrated. This was seen in 89% (25 of 28) of the insufficient cases, whereas false positive staining was seen in the remaining 11% (3 of 28) of the laboratories. The highest proportion of optimal results were seen on the Dako Autostainer and Leica Bond platforms, with 83% and 80%, respectively (see Table 3). In contrast only 50% and 48% were optimal on the Dako Omnis and Ventana BenchMark platforms.

59% (132 of 225) of the laboratories used Abs in RTU formats. This was an increase compared to the previous Podop assessment in 2012, where 42% of the laboratories used the RTU format. The most widely used RTU systems for Podop were the mAb clone D2-40 based **760-4395** from Ventana/Roche, intended for use on the Ventana BenchMark System, and the mAb clone D2-40 based **IR/IS072** from Dako/Agilent, intended for use on the Dako Autostainer System. Both RTU systems performed rather poorly. The proportion of sufficient results for 760-4395 and IR/IS072 were only 51% and 42%, respectively. The prevalent features of insufficient staining results were very different for the two systems. Using the 760-4395 system from Ventana/Roche, weak or false negative staining reactions were seen in 100% (32 of 32) of the insufficient cases, whereas false positive staining reactions were seen in all cases (15 of 15) of insufficient results with the Dako/Agilent IR/IS072 system.
22% (14 of 65) of the laboratories using the 760-4395 RTU followed the recommended protocol settings, but only 14% (2 of 14) of these laboratories achieved sufficient staining results and none were optimal. In contrast, 78% (51 of 65) of the laboratories modified protocol settings. Consequently, the proportion of sufficient and optimal staining results improved to 61% (31 of 51) and 27% (14 of 51), respectively (see Table 4). Typically, if the vendor recommended 2-step multimer detection system (UltraView) was substituted with sensitive 3-step multimer detection system (OptiView) including tyramide signal amplification, an optimal staining reaction could be achieved (see Figs. 7a – 9b). These data indicate a need for recalibration of the 760-4395 RTU system for the Ventana BenchMark platform and update of the vendor recommended protocol.
The IR/IS072 RTU for the Dako Autostainer performed surprisingly poorly in the current assessment, compared to the previous assessment (run 36 in 2012). In 2012 the pass rate was 100% (good or

optimal). In the current run, the general pass rate on the Dako Autostainer has dropped to 42%. No significant difference in pass rate was seen between laboratories using recommended or modified protocol settings (see Table 4). For IR/IS072 performed on the Dako Autostainer, the prevalent feature of an insufficient staining was false positive staining. The reason for this dramatic drop in pass rate is uncertain but might be related to a more challenging material circulated in the current run. In 15 cases, moderate to strong false positive staining reaction was seen in goblet cells in the appendix and in 4 of these cases an additional, weak to moderate false positive staining reaction was seen in neoplastic cells in the lung squamous carcinoma (tissue core no. 5). A typical context was seen; The stronger the staining of the goblet cells was, the more likely an additional and more critical false positivity in the lung squamous cell carcinoma was. Consequently, the normal goblet cells in appendix seem to be a useful and sensitive predictor of possible false positive staining reaction in the circulated lung squamous cell carcinoma.
14 laboratories using IR/IS072 with the recommended protocol settings on the Dako Autostainer provided very different staining results with respect of false positive staining. Irrespectively, that the same basic protocol settings were used 8 laboratories experienced moderate to strong false positivity and 6 laboratories did not (see Figs. 6a and 6b). The reason for these differences is uncertain. Lot to lot variation of IR/IS072 could be suspected, but the laboratory submitted data do not support this. Another explanation could be variation in the washing procedure (wash buffer, time and temperature) where data is not available in the submitted data set. A well-known observation on the Dako Autostainer platform is that the washing time in buffer (after primary Ab, link-Ab and polymer) varies with the number of slides stained in a given run. Running only a few slides typically results in short washing times, whereas a full run of 48 slides results in prolonged washing times (in total at least 10-15 min. longer). In theory, potential low affinity binding (cross reaction) to substances in goblet cells and neoplastic cells in the lung squamous cell carcinoma would be removed by prolonged washing. Supporting this theory is the fact that if IR/IS072 RTU was used within an LD-assay for Podop on the Dako Omnis - known for its efficient "high temperature" (32°C) washing - no false positive staining reaction was seen (see Figs. 5a and 5b). On the contrary, a high pass rate of 95% (18 of 19) was demonstrated with 58% (11 of 19) being optimal (see Table 1).

This was the third assessment of Podop in NordiQC (see Table 2). The number of participants increased from 102 laboratories in 2012 to 225 laboratories in the current assessment. Despite this 121% increase in participating laboratories, the pass rate from 2012 was slightly improved to 64% compared to 59% in run 36 in 2012.

Controls
Appendix is the recommended positive and negative tissue control for Podop. An at least moderate cytoplasmic staining of the Schwann cells in the muscularis propria of the appendix must be seen. In addition, endothelial cells of lymphatic vessels should be strongly stained, whereas no staining reaction should be seen in endothelial cells of the blood vessels and in the columnar epithelial cells and goblet cells of the mucosa.

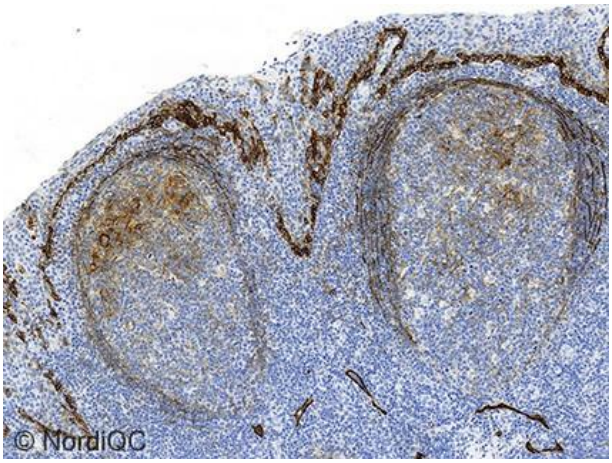


Fig. 1a (x100)
Optimal Podop staining reaction of the tonsil using a mAb clone D2-40 based LD-assay, optimally calibrated on the Dako Omnis. HIER was performed in TRS High pH (Dako/Agilent) for 24 min. at 97°C, with an incubation of the primary Ab for 30 min. at 1:75 dilution, using a 3-step polymer-based detection system (EnVision Flex+, Dako/Agilent). Strong staining of the lymphatic endothelial cells, the follicular dendritic cells in the germinal centres and the basal squamous epithelial cells is seen. Also compare with Figs. 2a-4a, same protocol.

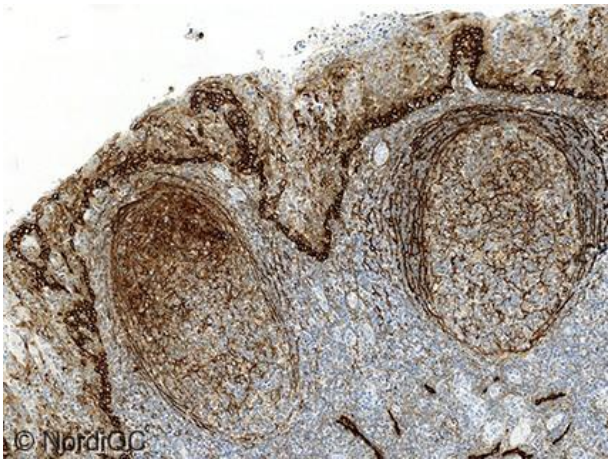


Fig. 1b (x100)
Insufficient Podop staining reaction of the tonsil using the mAb clone D2-40 based IR/IS072 RTU system on the Dako Autostainer platform with the protocol settings recommended by Dako/Agilent: HIER for 20 min. in TRS High pH (3-in-1), RTU Ab for 20 min. and 2-step polymer-based detection system (EnVision Flex). Very strong staining of the lymphatic endothelial cells, follicular dendritic cells in the germinal centres and the basal squamous epithelial cells is seen. But a general diffuse background staining results in a poor signal-to-noise ratio. Compare with Fig. 1a – same field. Also compare with Figs. 2b–4b, same protocol.

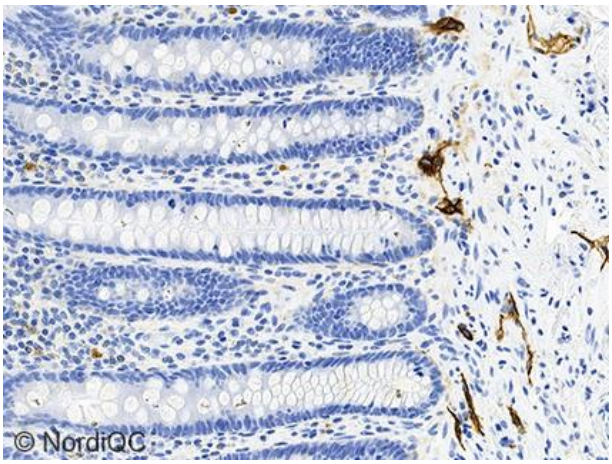


Fig. 2a (x200)
Optimal Podop staining reaction of the appendix using same protocol as in Fig. 1a. Strong staining of the lymphatic endothelial cells is seen, whereas columnar epithelial cells and goblet cells are negative. A high signal-to-noise ratio is obtained.

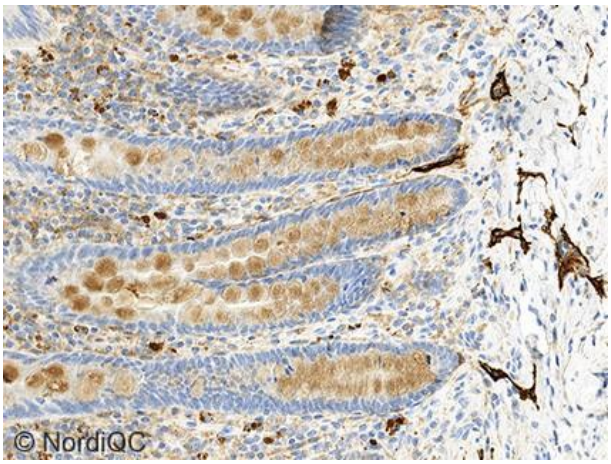


Fig. 2b (x200)
Insufficient Podop staining reaction of the appendix using same protocol as in Fig. 1b. Very strong staining of the lymphatic endothelial cells is seen, but at the same time false positive reaction is seen in virtually all goblet cells. Compare with Fig. 2a – same field. Also compare with Figs. 3b–4b, same protocol.

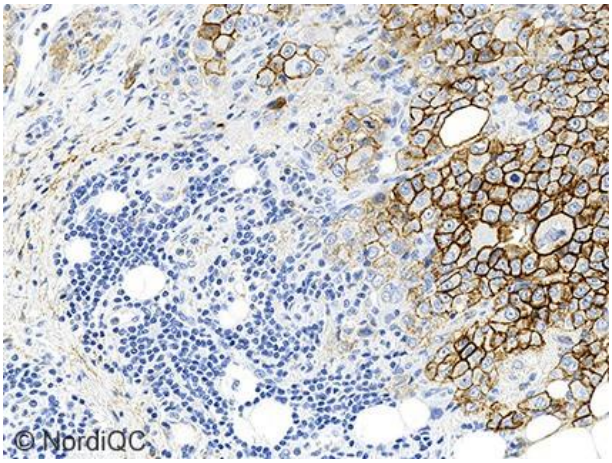


Fig. 3a (x200)
Optimal Podop staining reaction in the mesothelioma using same protocol as in Fig. 1a–2a. A strong, distinct predominantly membranous staining reaction of the neoplastic cells is seen. No background staining is seen, and a high signal-to-noise ratio is obtained.

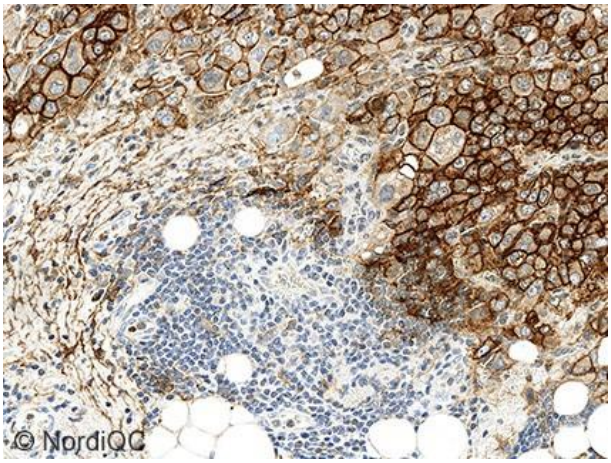


Fig. 3b (x200)
Insufficient Podop staining reaction in the mesothelioma using same protocol as in Fig. 1b–2b. A strong, predominantly membranous staining reaction of the neoplastic cells is seen, but the signal-to-noise is compromised by a weak, but general background staining. Compare with Fig. 3a – same field.

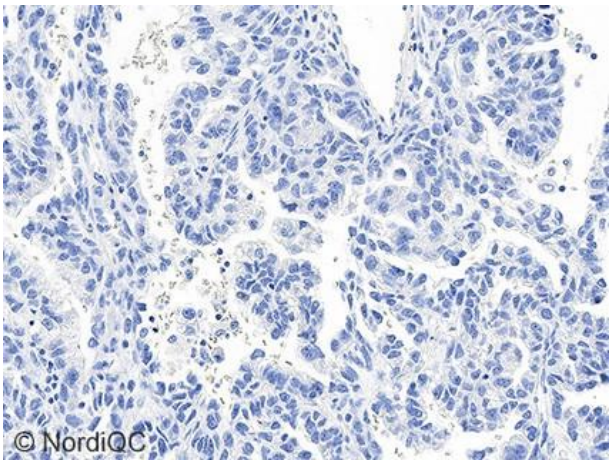


Fig. 4a (x200)
Optimal Podop staining reaction in the lung squamous cell carcinoma using same protocol as in Fig. 1a–3a. No staining reaction is seen in the neoplastic cells.

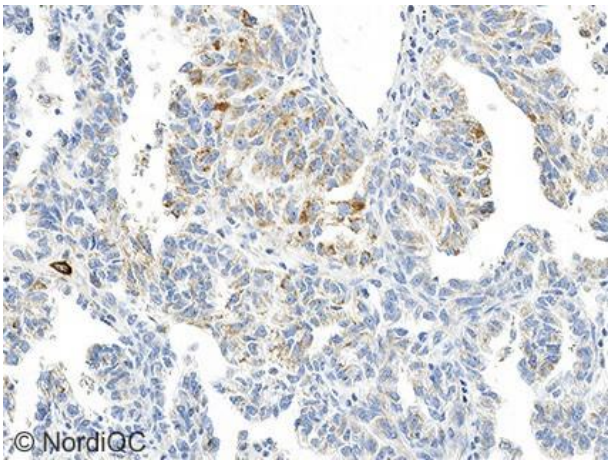


Fig. 4b (x200)
Insufficient Podop staining reaction in the lung squamous cell carcinoma using same protocol as in Fig. 1b–3b. False positive intracytoplasmic staining reaction is seen in the majority of neoplastic cells. Compare with Fig. 4a – same field. Also compare with Fig. 5b and 6b, same protocol.

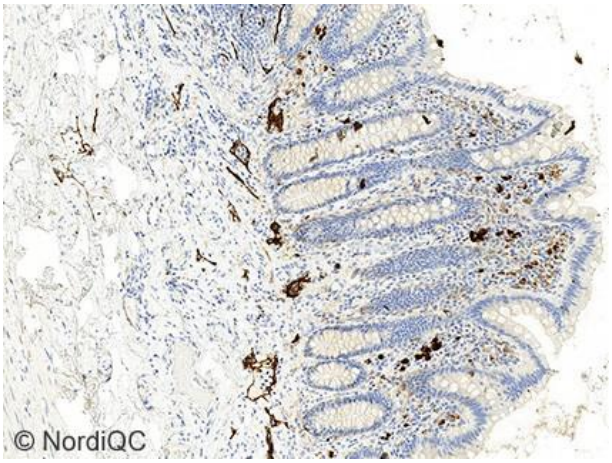


Fig. 5a (x100)
Optimal Podop staining reaction of the appendix using the mAb clone D2-40 based IR/IS072 RTU system intended for the Dako Autostainer platform but used on a Dako Omnis stainer with modified protocol settings: HIER for 20 min. in TRS High pH (3-in-1), RTU Ab for 20 min. and 3-step polymer-based detection system (EnVision Flex, Dako/Agilent). Compare with Fig. 5b.

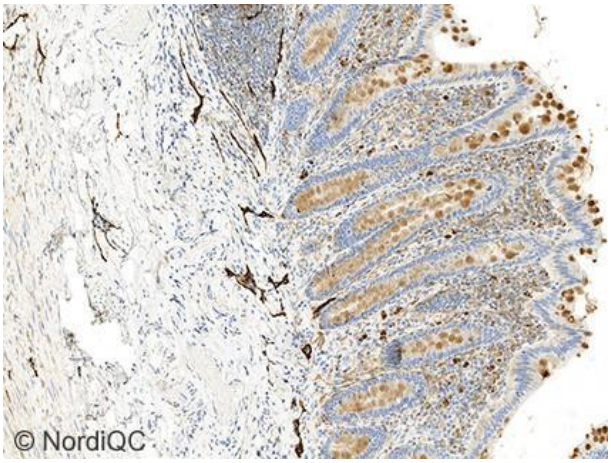


Fig. 5b (x100)
Insufficient Podop staining reaction of the appendix using the mAb clone D2-40 based IR/IS072 RTU system for the Dako Autostainer platform with the protocol settings recommended by Dako/Agilent; HIER for 20 min. in TRS High pH (3-in-1), RTU Ab for 20 min. and 2-step polymer-based detection system (EnVision Flex, Dako/Agilent). Compare with Fig. 5a – same field.

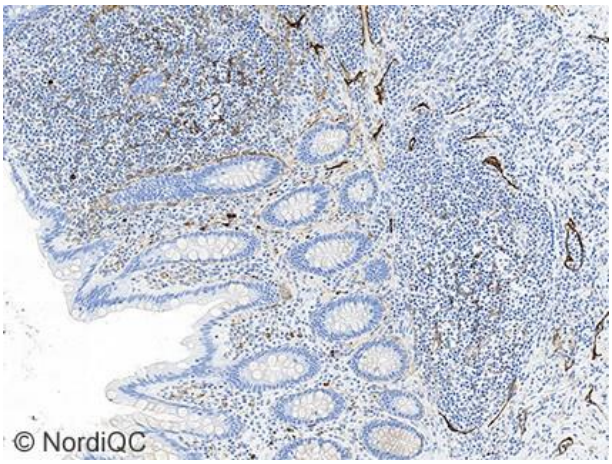


Fig. 6a (x100)
Optimal Podop staining reaction of the appendix using the mAb clone D2-40 based IR/IS072 RTU system on the Dako Autostainer platform with the protocol settings recommended by Dako/Agilent – same protocol settings as in Figs. 1b-6b. Despite identical basic protocol settings, no false positive staining reaction is seen in this case. Compare to Figs. 2b and 6b. The reason for the illustrated differences is unknown but might be related to variations in the washing procedure.

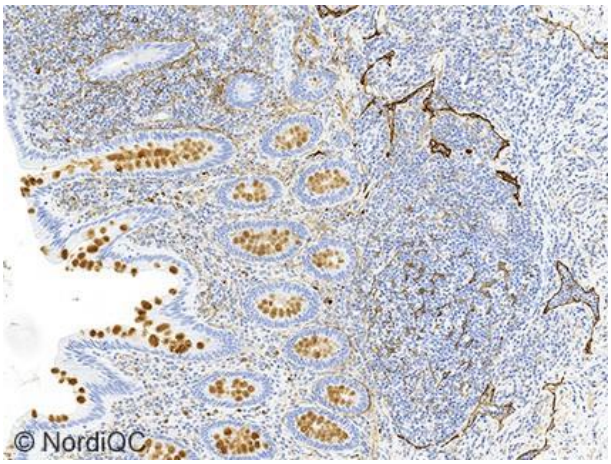


Fig. 6b (x100)
Insufficient Podop staining reaction of the appendix using the mAb clone D2-40 based IR/IS072 RTU system on the Dako Autostainer platform with the protocol settings recommended by Dako/Agilent – same protocol settings as in Fig. 2b and 6a. Similar to Fig. 2b, false positive reaction is seen in virtually all goblet cells. Compare to Fig. 6a where no false positive staining is seen – same field.

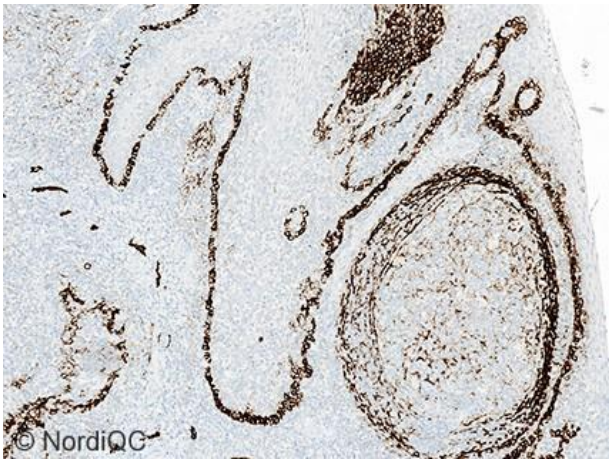


Fig. 7a (x100)
Optimal Podop staining reaction of the tonsil using a 760-4395 RTU (mAb clone D2-40) based LD-assay on the BenchMark Ultra. The vendor recommended protocol is modified, using prolonged incubation time in primary Ab and the highly sensitive OptiView with tyramide signal amplification as detection system. Strong staining of the lymphatic endothelial cells, follicular dendritic cells in the germinal centres and the basal squamous epithelial cells is seen. Also compare with Figs. 8a-9a, same protocol.

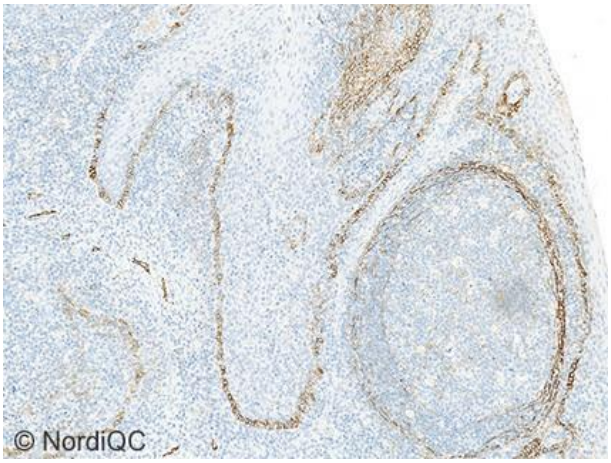


Fig. 7b (x100)
Insufficient Podop staining reaction of the tonsil using the mAb clone D2-40 based 760-4395 RTU-assay on the BenchMark Ultra. The vendor recommended protocol, based on the 2-step UltraView detection system, was followed. Weak to moderate staining of the lymphatic endothelial cells, the follicular dendritic cells in the germinal centres and the basal squamous epithelial cells is seen. Compare with Fig. 7a – same field. Also compare with Figs. 8b–9b, same protocol.

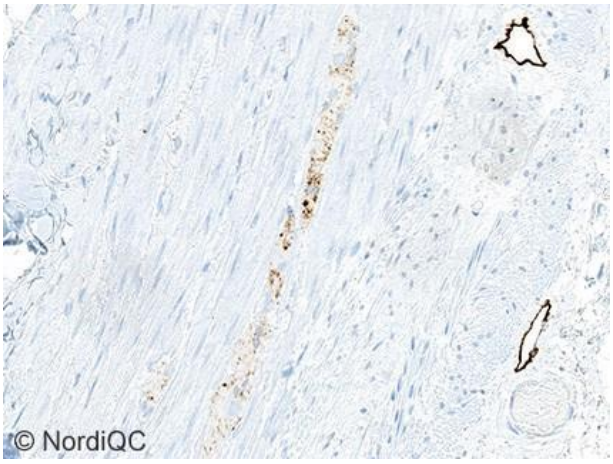


Fig. 8a (x200)
Optimal Podop staining reaction of the appendix using the same protocol as in Fig. 7a. An expected strong staining of the lymphatic endothelial cells is seen whereas the low-level Podop expressing Schwann cells in the muscularis propria shows a moderate staining.

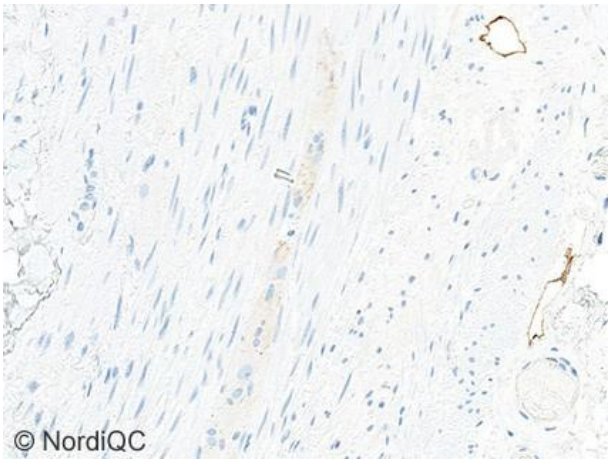


Fig. 8b (x200)
Insufficient Podop staining reaction of appendix using the same protocol as in Fig. 7b. Weak to moderate staining of the lymphatic endothelial cells is seen whereas the low-level Podop expressing Schwann cells in the muscularis propria are virtually negative. Compare with Fig. 8a – same field.

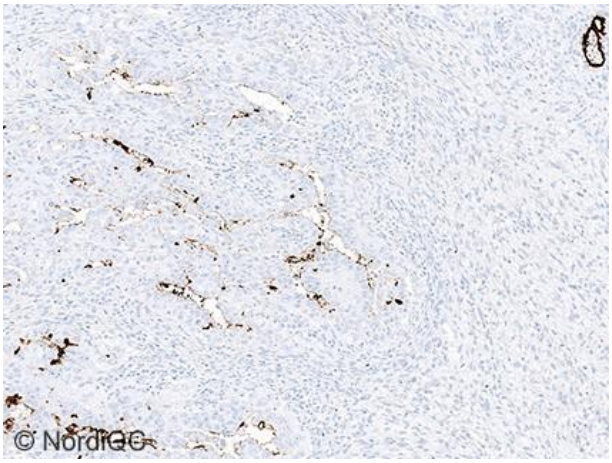


Fig. 9a (x100)
Optimal Podop staining reaction of the embryonal carcinoma using the same protocol as in Fig. 7a. An expected strong staining of the lymphatic endothelial cells is seen whereas a weak to moderate (apical) membranous staining reaction in the majority of the neoplastic cells is seen.

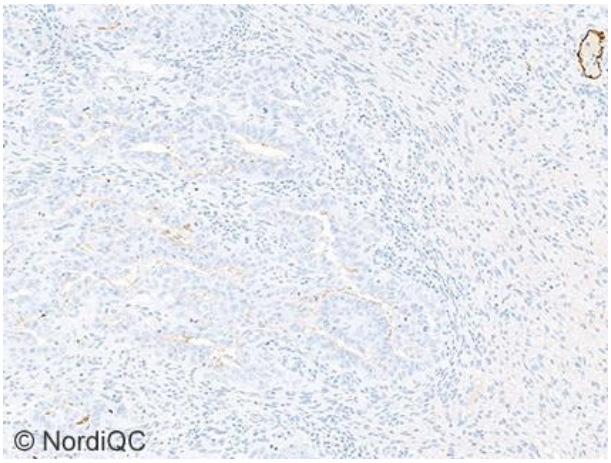


Fig. 9b (x100)
Insufficient Podop staining reaction of embryonal carcinoma using the same protocol as in Fig. 7b. Weak to moderate staining of the lymphatic endothelial cells is seen whereas the neoplastic cells are virtually negative. Compare with Fig. 9a – same field.

ON/LE/MV/RR 08.12.2018