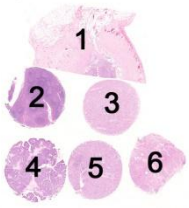
## Assessment Run 36 2012

# Podoplanin (Podop)

**Material**
The slide to be stained for Podop comprised:




1. Appendix, 2. Tonsil, 3. Seminoma, 4. Ovarian serous 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 moderate to strong, distinct predominantly cytoplasmic staining reaction of the fibroblastic cells and the Cajal 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.
- A strong, distinct predominantly membraneous staining reaction of the neoplastic cells in the seminoma and mesothelioma.
- A weak to moderate staining reaction of the majority of the neoplastic cells in the serous ovarian carcinoma.
- A negative staining reaction of the neoplastic cells of the lung squamous cell carcinoma.

102 laboratories participated in this assessment. 59 % achieved a sufficient mark (optimal or good). Table 1 summarizes used antibodies (Abs) and assessment marks.

Table 1. **Antibodies and assessment marks for Podop, run 36**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **D2-40** | 48<br>3<br>2<br>1<br>1<br>1 | Dako<br>Signet<br>Biocare<br>Cell Marque<br>Immunologic<br>Zytomed | 10 | 20 | 22 | 4 | 54 % | 56 % |
| mAb clone **AB3** | 1 | AngioBio | 0 | 1 | 0 | 0 | - | - |
| mAb clone **18H51** | 1 | Acris | 0 | 0 | 0 | 1 | - | - |
| rmAb clone **EP215** | 1 | Epitomics | 0 | 0 | 1 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **D2-40 IS/IR072** | 15 | Dako | 11 | 4 | 0 | 0 | 100 % | 100 % |
| mAb clone **D2-40 N1607** | 3 | Dako | 0 | 3 | 0 | 0 | - | - |
| mAb clone **D2-40 760-4395** | 21 | Ventana/Cell Marque | 0 | 8 | 13 | 0 | 38 % | - |
| mAb clone **D2-40 322M-17/18** | 2 | Cell Marque | 0 | 1 | 1 | 0 | - | - |
| mAb clone **D2-40 MON-RTU1092** | 1 | Monosan | 0 | 1 | 0 | 0 | - | - |
| mAb clone **D2-40 MAD-000402QD** | 1 | Master Diagnostica | 0 | 1 | 0 | 0 | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Total | 102 | 21 | 39 | 37 | 5 | - | |
| Proportion | | 21 % | 38 % | 36 % | 5 % | 59 % | |

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

Detailed analyzes of Podop, Run 36
Following protocol parameters were central to obtain an optimal staining:

**Concentrated antibodies**
mAb clone **D2-40**: Protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS High pH;Dako) or TRS pH 9 (4/9)*, Bond Epitope Retrieval Solution 2 (BERS2;, Leica) (2/6),Cell Conditioning 1 (CC1; Ventana) (2/23), Tris-EDTA/EGTA pH 9 (1/10) or Target Retrieval Solution pH 6.1 (TRS Low pH;Dako) (1/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:10-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 20 out of 36 (56 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

**Ready-To-Use antibodies**
mAb clone **D2-40** (prod. no. IS/IR071, Dako): The protocols giving an optimal result were based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (heating time 10-20 min at 95-100°C),20-30 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Based on these protocol settings 15 of 15 (100 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody
- Less successful performance of the mAb clone D2-40 on the Ventana BenchMark platform

In concordance with previous NordiQC assessments, the prominent feature of an insufficient staining result was a general too weak or false negative staining of the structures expected to be demonstrated. Virtually all the participating laboratories were able to demonstrate Podop in the neoplastic cells of the mesothelioma and the seminoma, whereas the demonstration of Podop in the lymphatic endothelial cells, the Cajal cells of the appendix and in particular the follicular dendritic cells in the germinal centres of the tonsil were more challenging.

All participants except one used HIER as pre-treatment.
20 of 21 protocols producing optimal results were based on HIER using an alkaline HIER buffer. Sufficient result could be obtained with the mAb clones D2-40 and AB3. Pass rates of the mAb clone D2-40 was highly influenced by the stainer platform used: less successful performance was seen on the Ventana BenchMark platform with both concentrates and RTU formats, since only 19 of 50 (38%) laboratories using this platform obtained a sufficient staining result (2 (4%) of these were assessed as optimal). If the mAb clone D2-40 was applied as concentrate or RTU format by non-Ventana users, 39 of 46 laboratories obtained a sufficient staining result (85%) of which 18 (39%) were assessed as optimal.

The most successful and robust assay for Podop was a RTU system (Dako) based on the mAb clone D2-40 (IS/IR071) giving a pass rate of 100 % out of which 73 % (11 out 15 laboratories) were assessed as optimal. Using the RTU format according to recommendations from the vendor, consistent staining for Podob was observed in cellular structures with a high antigen expression (lymphatic endothelial cells) but also in cells with a lower antigen expression (Cajal cells of the appendix and follicular dendritic cells in germinal centres of the tonsil). In particular, the demonstration of Podop in cells with low antigen expression was superior compared to other RTU systems based using the same clone.

**Controls**
Appendiceal tissue was applicable as a critical staining quality indicator for Podop. An at least moderate cytoplasmic staining of Cajal 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.

Effect of external quality assessment
This was the 2th assessment of Podop in NordiQC. A small decrease in the proportion of sufficient results was seen compared to the previous run in 2007, see table 2.

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

| | Run 19 2007 | Run 36 2012 |
|---|---|---|
| Participants, n= | 29 | 102 |
| Sufficient results | 69 % | 59 % |

The low pass rate (59%) in this run might be explained by several factors including a large proportion of new participants and more challenging tissue material circulated. However, the less successful performance of the mAb clone D2-40 on the Ventana BenchMark platform seemed to have a significant impact. 49% of the laboratories used this platform and for this group an overall pass rate of only 38 % was seen.

**Conclusion**

The mAb clone **D2-40** is a recommendable Ab for Podob. Efficient HIER in an alkaline buffer in combination with a sensitive and specific IHC system is mandatory for optimal performance. The performances of the Ab were influenced by the stainer platform. On non-Ventana platforms the mAb clone D2-40 both as concentrate and as RTU format gave the highest proportion of sufficient results. In this assessment the Ready-To-Use system based on the mAb clone **D2-40** from Dako was superior compared to other systems with a pass rate of 100% of which 73% were assessed as optimal.
Normal appendix is an appropriate control: The Cajal cells must show an at least moderate cytoplasmic staining reaction, while the lymphatic endothelial cells should be strongly stained.

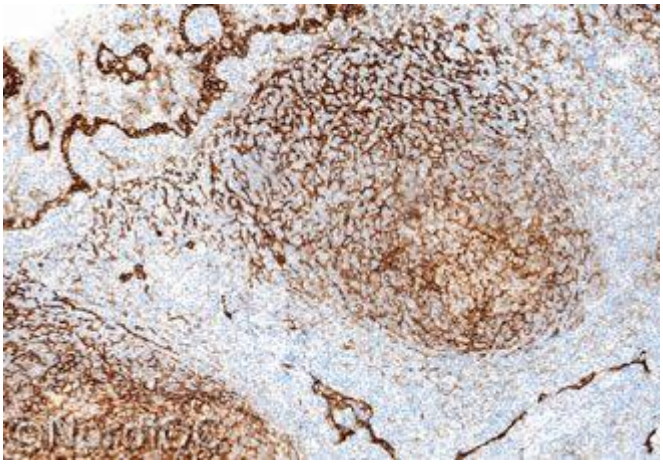


Fig. 1a
Optimal staining for Podop of the tonsil using the mAb clone D2-40 optimally calibrated as a concentrate, HIER in BERS2, Leica and a 3-step polymer based detection system. The basal squamous epithelial cells and the lymphatic endothelial cells show a strong cytoplasmic staining reaction, while a moderate staining reaction is seen in the follicular dendritic cells in the germinal centres.

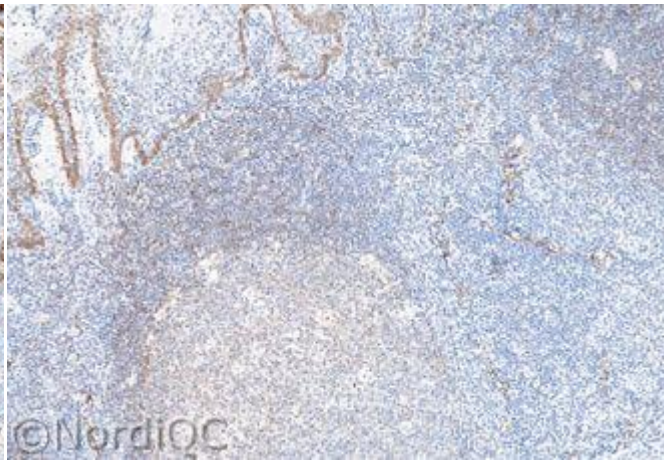



Fig. 1b
Insufficient staining for Podop of the tonsil using the mAb clone D2-40 as a concentrate with a too low sensitivity (too low conc. of the primary Ab and HIER in citrate pH 6).
Only a weak cytoplasmic staining reaction is demonstrated in the cells with a high antigen expression as the basal squamous epithelial cells, whereas no staining is seen in the follicular dendritic cells – compare with Fig. 1a.

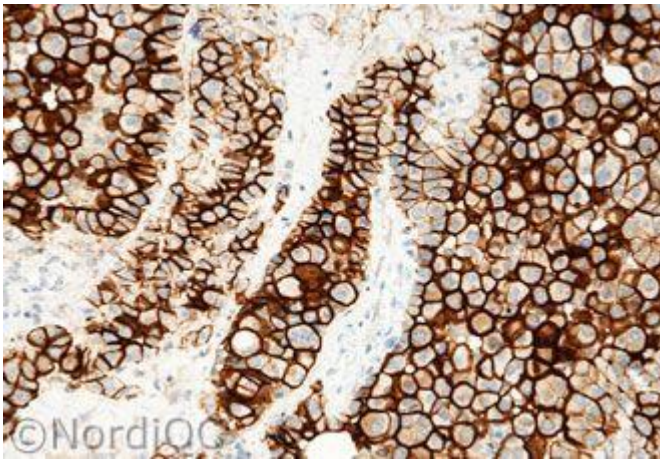



Fig. 2a
Optimal staining for Podop of the mesothelioma using the same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct membranous staining reaction.

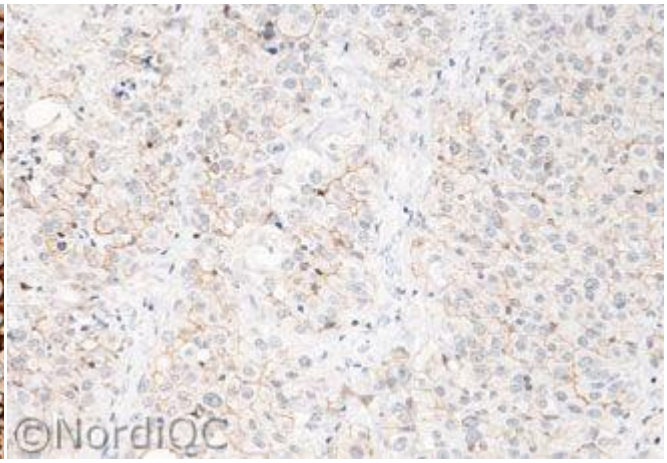



Fig. 2b
Insufficient staining for Podop of the mesothelioma using the same protocol as in Fig. 1b. The proportion and the intensity of the cells demonstrated is significantly reduced – compare with Fig. 2a.

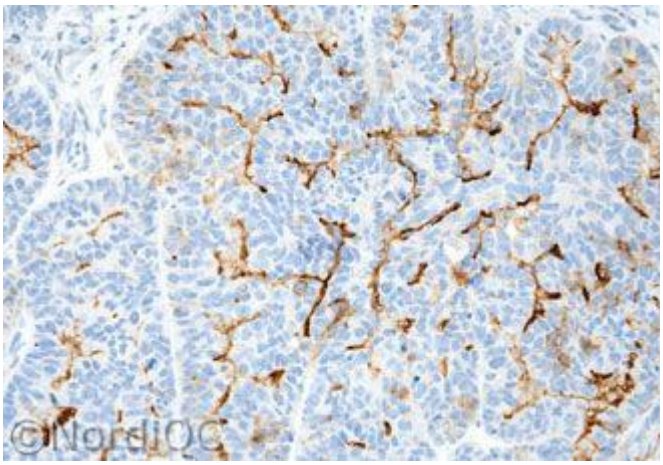



Fig. 3a
Optimal staining for Podop of the ovarian serous carcinoma using the same protocol as in Figs. 1a & 2a. The majority of the neoplastic cells show a moderate to strong distinct membranous staining reaction.

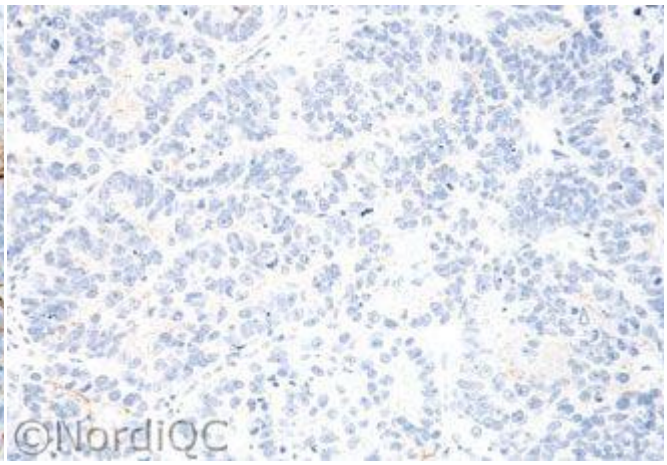



Fig. 3b
Insufficient staining for Podopl of the ovarian serous carcinoma using the same protocol as in Figs. 1b & 2b. Only scattered neoplastic cells show a weak and patchy membranous staining reaction - compare with Fig.3a.

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