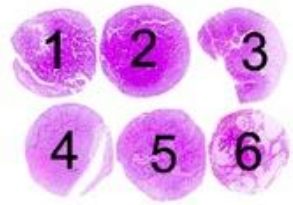
Assessment Run 19 2007

Podoplanin (Podop)



The slide to be stained for Podoplanin (Podop) comprised:
1. Ovarian clear cell carcinoma, 2. Ovarian serous carcinoma, 3. Appendix, 4. Seminoma, 5. Embryonal carcinoma, 6. Malignant mesothelioma, epithelioid.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Podop staining as optimal included:

- A strong, distinct predominantly cytoplasmic staining of the lymphatic endothelial cells and the Cajal cells of the muscularis propria in the appendix.
- A strong, distinct predominantly cytoplasmic staining of the majority of neoplastic cells of the seminoma.
- A strong, distinct predominantly membranous staining of majority of the neoplastic cells in the malignant mesothelioma and the embryonal carcinoma.
- No staining of the neoplastic cells of the ovarian serous carcinoma and endothelial cells in blood vessels.

29 laboratories submitted stains. At the assessment 9 achieved optimal marks (31 %), 11 good (38 %), 8 borderline (28 %) and 1 poor mark (3 %).

The following Abs were used:
mAb clone **18H4** (Acris, n=2)
mAb clone **D2-40** (Dako, n=20; Signet, n=4; BioCare, n=1; ID-Labs Inc.; n=1, Serotec, n=1)

Optimal staining for Podop in this assessment was only obtained with the mAb clone **D2-40** (9 out of 27, 33%).

Using the mAb clone **D2-40** the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) typically with the alkaline buffer Tris-EDTA/EGTA pH 9, but also HIER in Citrate pH 6,0 or Target Retrieval Solution pH 6.1 (Dako S1699) could be used to obtain an optimal result. The mAb was typically diluted in the range of 1:25 – 1:100 depending on the total sensitivity of the protocol employed. Using these settings 20 out of 22 (91 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient staining were (often seen in combination):
- Too low concentration of the primary Ab
- Less successful primary Ab
- HIER in other buffer than Tris-EDTA/EGTA pH 9, Citrate pH 6,0 or Target Retrieval Solution pH 6.1

In this assessment the prevalent feature of the insufficient result was a too weak staining of the neoplastic cells of the embryonal carcinoma and the lymphatic endothelial cells, whereas virtually all laboratories could demonstrate Podop in the neoplastic cells of the seminoma and the mesothelioma. In all the optimal results the Cajal cells showed a distinct reaction, while these cells were negative or only showed a dubious reaction in the insufficient results, indicating that the Cajal cells can be useful as a critical quality indicator for the immunohistochemical demonstration of Podop.
In a few cases of using D2-40 in a high concentration combined with a sensitive protocol based on efficient HIER a nuclear reaction of stromal cells was observed in the circumference of the seminoma. This is probably due to an absorption of the antigen diffused from the seminoma cells. No nuclear reaction was observed in other tissues.

Conclusion
The mAb clone **D2-40** appears to be a useful Ab for Podop. HIER is mandatory to obtain an optimal result. Normal appendix is useful as control tissue: Lymphatic endothelial cells and Cajal cells shall show a distinct staining. No staining shall be seen in non-lymphatic endothelial cells and smooth muscle cells.

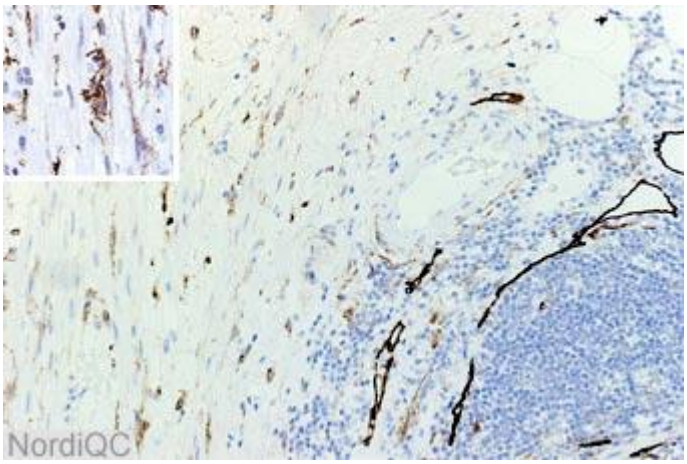


Fig. 1a
Optimal staining for Podop. in the appendix.
The endothelial cells lining the lymphatic vessels show a strong and distinct staining. In the tunica muscularis the Cajal cells are clearly demonstrated – Insert high magnification x40 of the tunica muscularis.

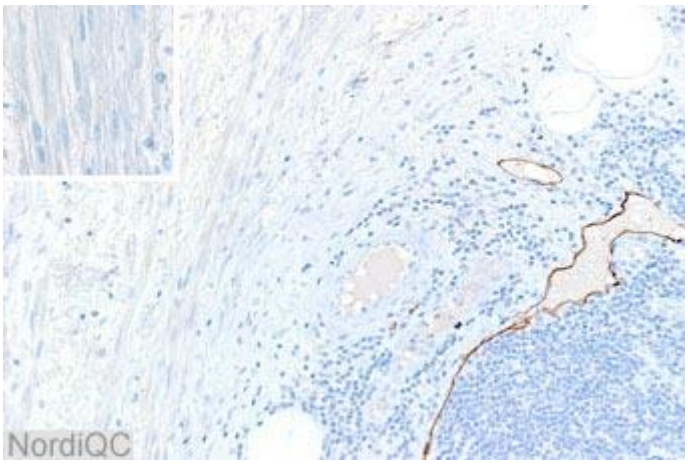


Fig. 1b
Staining for Podop in the appendix using an insufficient protocol (same fields as in Fig 1a).
The lymphatic endothelial cells only show a weak to moderate staining and the Cajal cells are completely negative – insert high magnification x40 of the tunica muscularis. Also compare Fig. 3a and 3b.

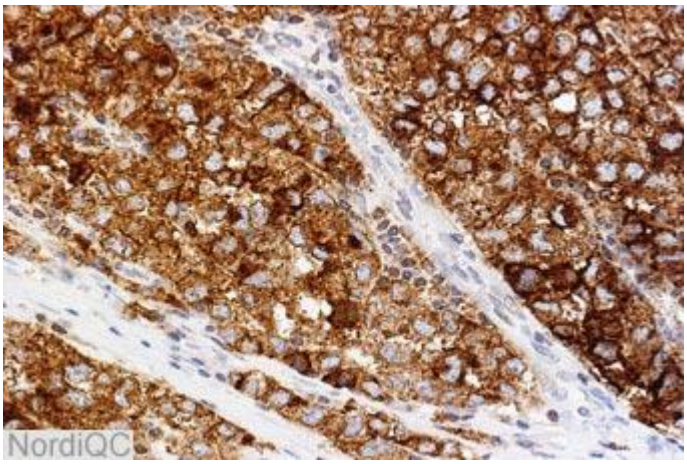


Fig. 2a
Optimal staining for Podop. of the seminoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining with a focal dot like reaction.

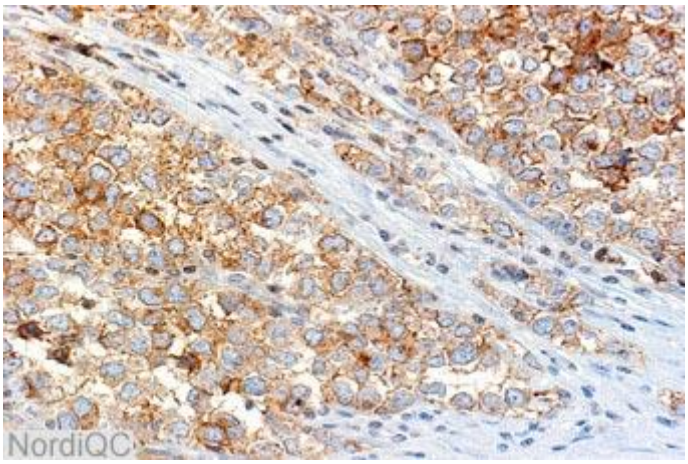


Fig. 2b
Staining for Podop. of the seminoma using same insufficient protocol as in Fig. 1b and 3b. The neoplastic cells show a weak to moderate cytoplasmic staining and a focal dot like reaction. However compare with Fig. 3b.

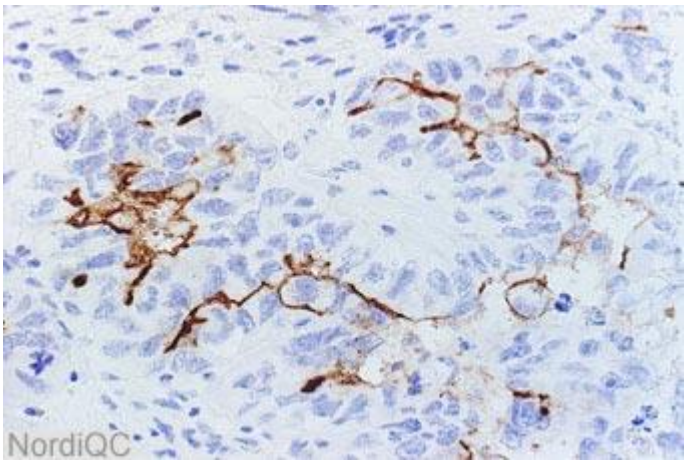


Fig. 3a
Optimal staining for Podop. of the embryonal carcinoma using same protocol as in Fig. 1a. The majority of the neoplastic cells show a distinct membranous staining.

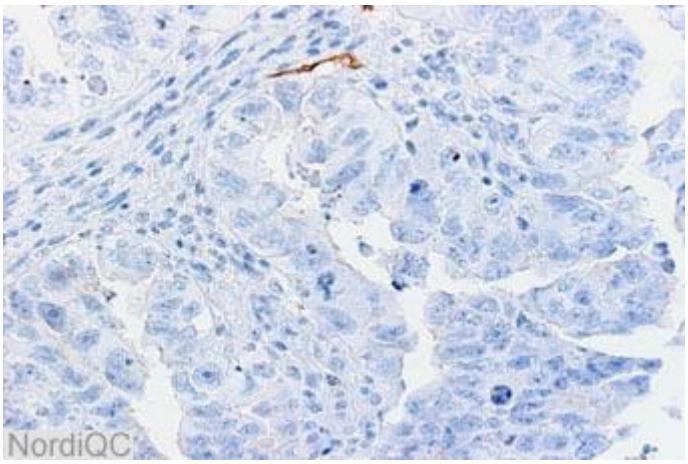


Fig. 3b
Insufficient staining for Podop. of the embryonal carcinoma using same insufficient protocol as in Fig. 1b and 2b.. The neoplastic cells are false negative, while only a lymphatic vessel is demonstrated.

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