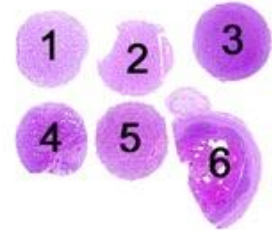
NordiQC

Assessment Run 24 2008

Placental Alkaline Phosphatase (PLAP)

The slide to be stained for Placental Alkaline Phosphatase (PLAP) comprised:
1-2. Testis with intratubular germinal cell neoplasia (IGCN), 3. Seminoma,
4. Embryonal carcinoma, 5. Placenta, 6. Appendix.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a PLAP staining as optimal included:

- A strong and distinct predominantly membranous but also cytoplasmic reaction of the majority of the neoplastic cells of the two IGCNs, the seminoma and the embryonal carcinoma.
- A strong predominantly membranous but also cytoplasmic staining of the cytotrophoblastic and syncytiotrophoblastic cells in the placenta with no or only minimal reaction in the stromal cells.
- Negative staining of all other cells.

115 laboratories submitted stains. At the assessment 3 achieved optimal marks (3 %), 57 good (50 %), 41 borderline (36 %) and 14 poor marks (12 %).

The following Abs were used:
mAb clone **PL8-F6** (BioGenex, n=36)
mAb clone **8A9** (Dako, n=44; Novocastra/Leica, n=8)
mAb clone **NB10** (Ventana, n=12)
mAb clone **8B6** (Dako, n=1)
rmAb clone **SP15** (NeoMarkers/Thermo, n=8)
pAb **A0268** (Dako, n=6)

Optimal staining for PLAP in this assessment was only obtained with the mAb **PL8-F6** (3 out of 36).

PL8-F6: All three optimal protocols were based on heat induced epitope retrieval (HIER) using an alkaline buffer: Tris-EDTA/EGTA (2/21)* or Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/2).
The mAb was diluted in the range of 1:100 – 1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 19 out of 22 (86 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

The low proportion of optimal results using the mAb clone PL8-F6 (8%) compared to the relative high pass rate of 86% of laboratories using the Ab was due to the Mouse Ascites Golgi (MAG) reaction observed with the format as an ascites Ab of this clone. For yet unexplained reasons the MAG reaction was not seen with the lot MU2280600 used by 3 laboratories, whereas it was observed in all other lots. The other protocol settings (HIER, Ab titer and detection system) used by these 3 laboratories were similar to the laboratories getting the MAG reaction. NordiQC has contacted BioGenex to identify the reason for the different reaction patterns but not got an answer yet.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary Ab
- Omission of HIER
- Less successful Abs (7/8 protocols using SP15 were insufficient)
- Less successful RTU Ab clone NB10 (7/12 were insufficient)

In this assessment the prevalent feature of an insufficient staining was a too weak or completely negative reaction of the neoplastic cells in the three different germinal cell tumours and in particular the neoplastic cells of the IGCNs. It was also observed that the two most popular Abs, clone 8A9 and PL8-F6 both gave an undesired cross reactivity. The clone 8A9 gave a distinct cytoplasmic cross reaction with smooth muscle cells in all the tissues included in the block for the assessment, whereas the clone PL8-F6 gave a dot-like cytoplasmic reaction (MAG) in the epithelial cells of the appendix (which was obtained from a patient with blood group A. The cross reaction of the mAb clone 8A9 was also addressed in the last assessment, and the laboratories seem to try to reduce the reaction by e.g. decreasing the concentration of the Ab. but this also reduces the sensitivity and only

35% (18/52) using 8A9 obtained a sufficient result.
It was also observed that none of the other Abs used could be used to obtain an optimal result and especially the rmAB clone SP15 was less successful as 7/8 laboratories using this Ab obtained an insufficient mark. The vendor recommends no retrieval should be performed for the Ab, but all protocols omitting retrieval gave an insufficient result as the IGCN was negative or too weak. If HIER was performed the signal-to-noise ratio was compromised and both a false positive cytoplasmic reaction and nuclear reaction was seen in mature normal spermatocytes. Finally when a RTU Ab is used, it is vital that the protocol settings for the IHC procedure is adjusted carefully and in this assessment 7/12 laboratories used protocol settings giving a too low sensitivity.

This was the second assessment of PLAP, and the proportion of sufficient results declined to 52% in this run compared to 63% in run 14, 2005. The lower pass rate is probably due to a combination of an increase in the number of participants (from 77 in run 14 to 115 in this run) and more challenging tissue material circulated.

It was difficult to use placenta as a recommendable control for PLAP, as this mainly could give information about the sensitivity but not about the specificity. Regarding the sensitivity the cytotrophoblastic and syncytiotrophoblastic cells in the placenta should give an as strong as possible staining with minimal reaction in the stromal cells and to evaluate any unspecific reaction appendix, preferable blood type A, should be used.

Conclusion
The mAb clone PL8-F6 was the only Ab that could give an optimal result. HIER in alkaline buffer was mandatory. Caution should be taken when the Ab is used, as the ascites format of the Ab gives cross reaction in blood type A tissue. Due to the many difficulties with the used PLAP Abs, the laboratories should consider to use other Abs such as OCT 3/4.

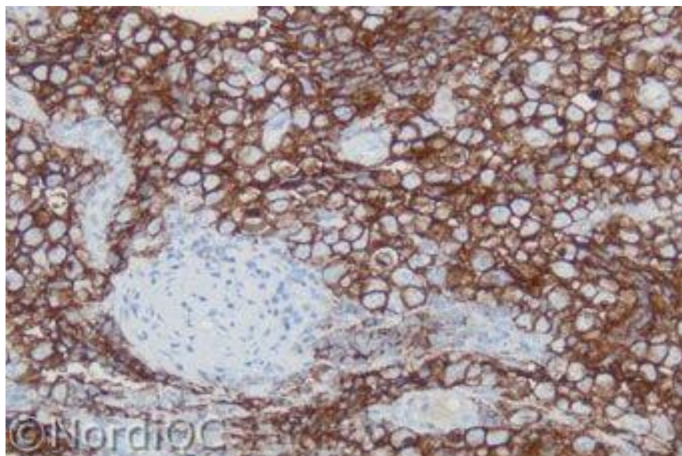


Fig. 1a
Optimal staining for PLAP of the seminoma using the mAb clone PL8-F6 with HIER.
Virtually all the neoplastic cells show a distinct and strong, predominantly membranous staining.

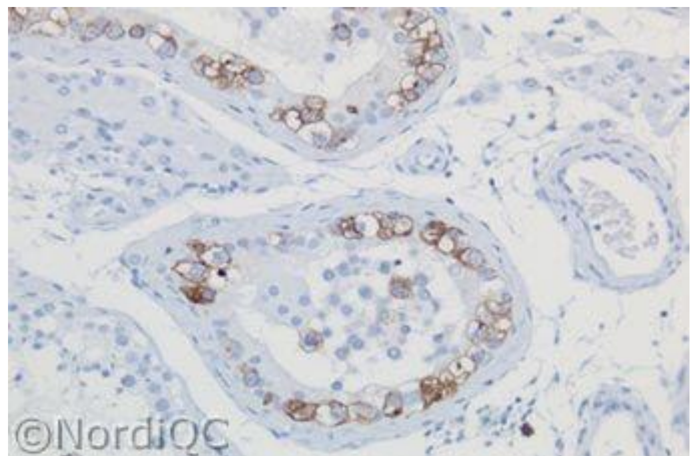
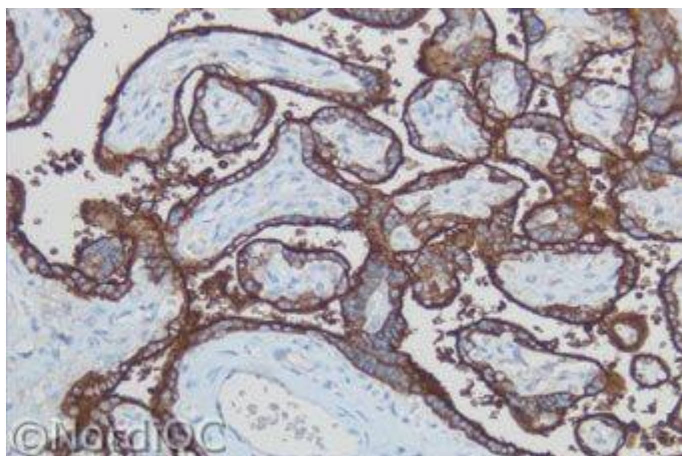


Fig. 1b
Optimal staining for PLAP of the IGCN using same protocol as in Fig. 1a.
The majority of the neoplastic cells show a moderate to strong and distinct membranous staining.



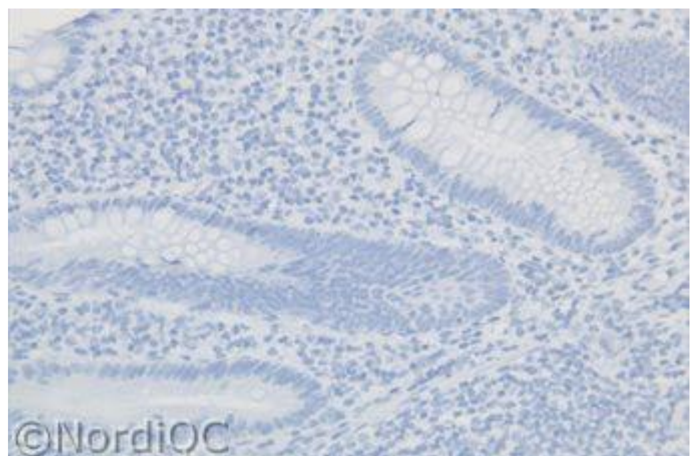
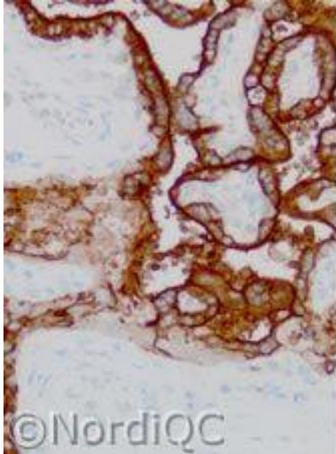
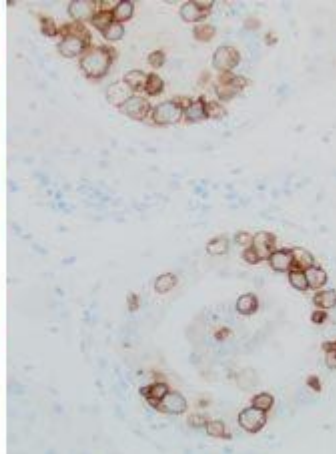


Fig. 2a
Optimal staining for PLAP of the placenta using same protocol as in Fig. 1a.
All the trophoblastic cells are strongly stained.

Fig. 2b
Optimal staining for PLAP of the appendix blood type A using same protocol as in Fig. 1a.
No reaction is seen – of particular interest no MAG reaction is seen in the epithelial cells.
Also compare with Fig. 3b – same mAb clone.





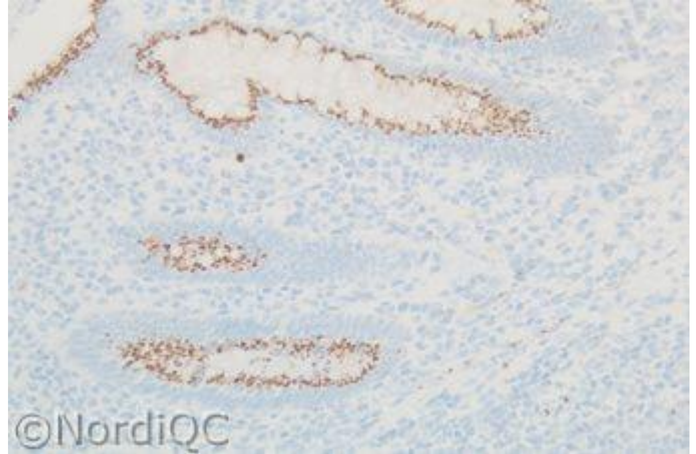
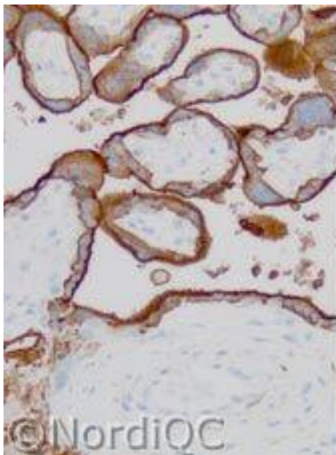
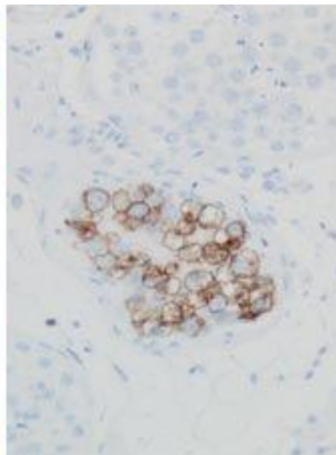


Fig. 3a
Staining for PLAP using the mAb clone PL8-F6 with HIER assessed as good. See also Fig. 3b.
Left: Placenta. All the trophoblastic cells are strongly stained.
Right: IGCN. The majority of the neoplastic cells show a moderate to strong and distinct membranous staining.

Fig. 3b
Staining for PLAP of the appendix blood type A assessed as good using same protocol as in Fig. 3a.
The epithelial cells show a false positive MAG reaction.
No protocol parameter can yet be identified causing the different reaction pattern in the appendix, when same mAb clone and similar protocol settings were used.





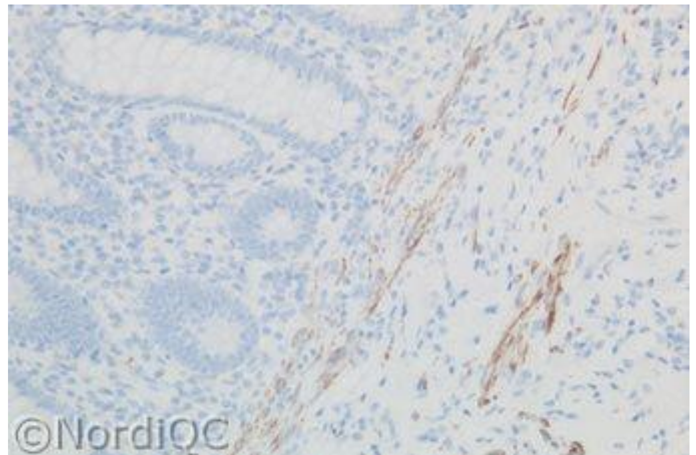


Fig. 4a
Staining for PLAP using the mAb clone 8A9 with HIER assessed as good. See also Fig. 4b.
Left: Placenta. All the trophoblastic cells are strongly stained.
Right: IGCN. The majority of the neoplastic cells show a moderate to strong and distinct membranous staining.

Fig. 4b
Staining for PLAP of the appendix blood type A assessed as good using same protocol as in Fig. 4a.
No staining is seen in the epithelial cells, but a moderate and distinct cytoplasmic staining is seen in the smooth muscle cells.

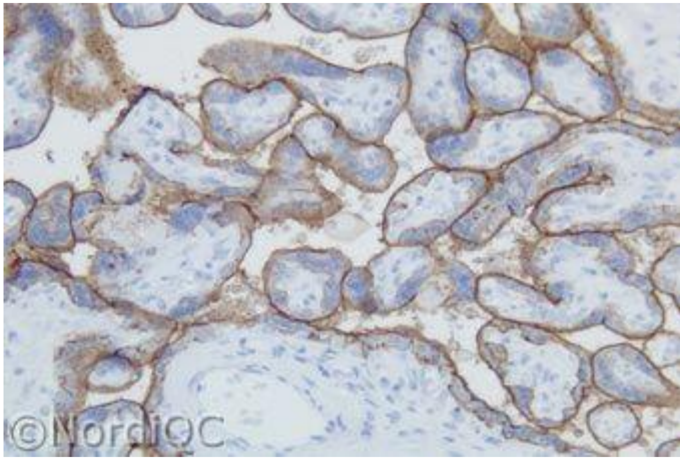


Fig. 5a
Insufficient staining for PLAP in the placenta, using a too dilute primary Ab.
The trophoblastic cells are only weakly demonstrated – same field as in Fig. 2a.

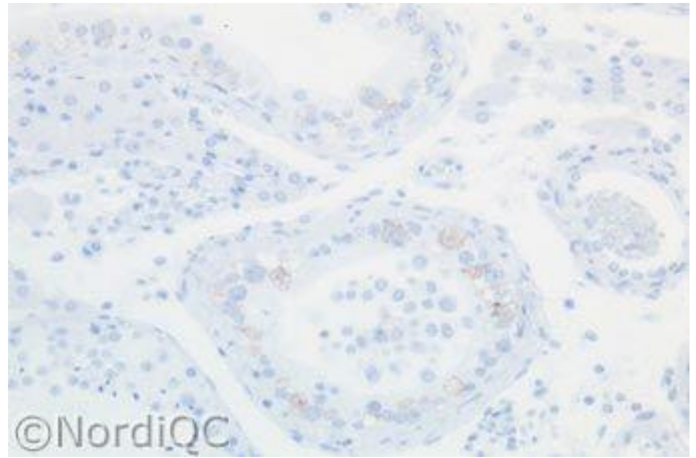


Fig. 5b
Insufficient staining for PLAP of the IGCN using same protocol as in Fig. 5a.
The neoplastic cells only show a weak and dubious staining – same field as in Fig. 1b.

SN/HN/MV/LE 8-12-2008