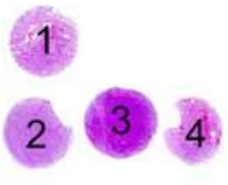
Assessment Run 11 2004

Human chorionic gonadotropin (HCG)

The slide to be stained for human chorionic gonadotropin (HCG) comprised:
1. Placenta (16 weeks), 2. Seminoma, 3. Embryonal carcinoma, 4. Choriocarcinoma.



Criteria for assessing a HCG staining as optimal included: A strong and distinct cytoplasmic staining of the normal placental throphoblastic cells, the trophoblastic cells of the choriocarcinoma (scarsely represented in some sections) and the syncytiotrophoblast like cells of the seminoma, whereas the neoplastic cells of the seminoma and the embryonal carcinoma should be negative or only weakly stained.

61 laboratories submitted a HCG staining. At the assessment 24 achieved optimal staining (39 %), 17 good (28 %), 14 borderline (23 %) and 6 (10 %) poor staining.

The following Abs were used:
pAb A0231 (DakoCytomation; n=55)
pAb 760-2650 (Ventana; n=2)
pAb NCL-HCGp (Novocastra; n=1)
mAb clone 2B1.3 (Immunotech; n=1)
mAb clone ZSH 17 (Zymed; n=1)

In this assessment an optimal staining could only be obtained with the pAbs A0231 and NCL-HCGp.

Using the pAb A0231 the optimal result could be obtained both with HIER and proteolytic pre-treatment. The choice of HIER buffer or proteolytic enzyme had no obvious influence. The main parameter seemed to be a correct calibrated primary Ab dilution: In the optimal protocols A0231 was diluted in the range of 1:1.000 – 12.000 using proteolytic pre-treatment and 1:3.000 – 50.000 using HIER.
The pAb NCL-HCGp was used with proteolytic pre-treatment and diluted 1:350.
The listed dilutions were depending on the total sensitivity of the used protocols.

The prevalent feature of the insufficient staining was a moderate or strong non-specific staining of especially the neoplastic cells of the seminoma and the stroma of the placenta (a slight background reaction in these two specimens was accepted). Only 2 out of 20 protocols giving an insufficient staining revealed a too weak or false negative reaction. Both these protocols were without pre-treatment.

The most frequent causes of insufficient staining were:
- Too high concentration of the primary antibody
- No epitope retrieval
- Inappropriate choice of primary Ab

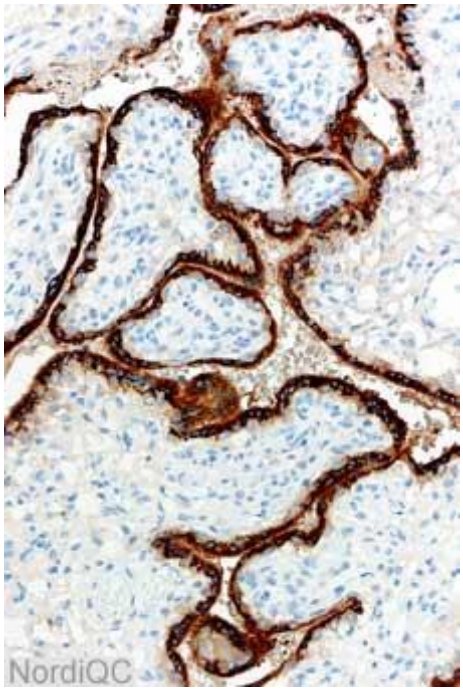


Fig. 1a
Optimal HCG staining of placenta. All trophoblastic cells are strongly stained.

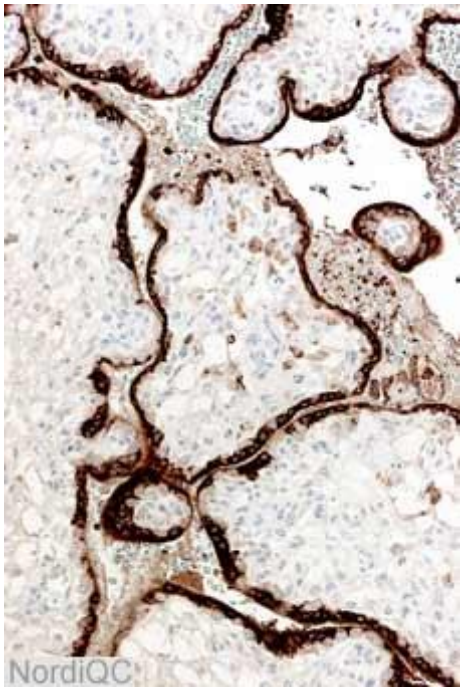


Fig. 1b
Optimal HCG staining of placenta. All trophoblastic cells are strongly stained. The Hofbauer cells are also stained, and a faint stromal reaction is seen.

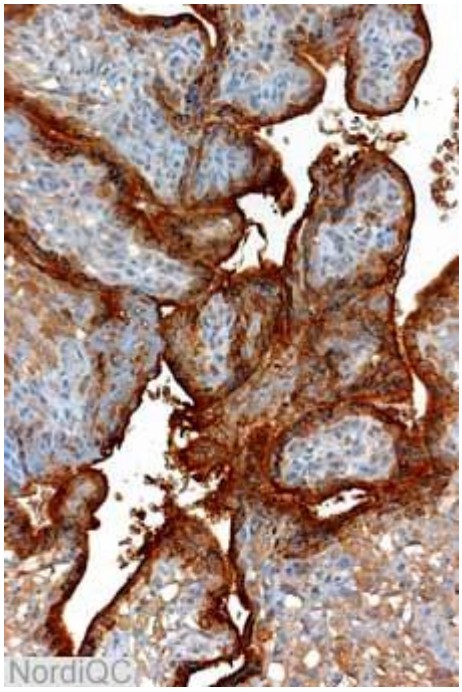


Fig. 1c
Insufficient HCG staining of placenta. The trophoblastic cells are strongly stained but also a moderate stromal reaction is seen. Also compare Fig. 2c.

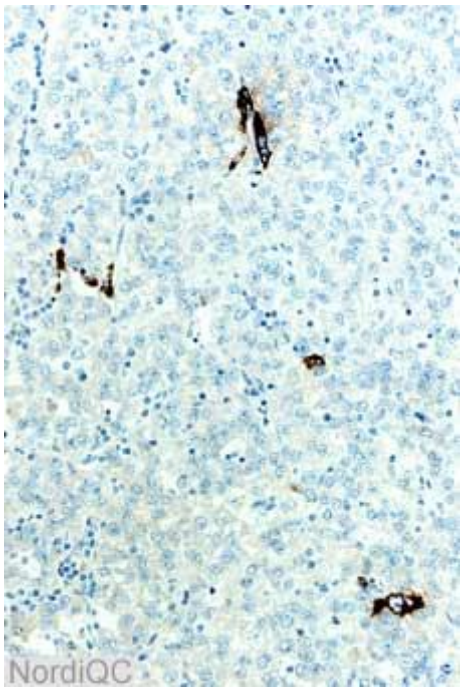


Fig. 2a
Optimal HCG staining of the seminoma. The trophoblast like cells are strongly stained while the neoplastic cells are unstained.

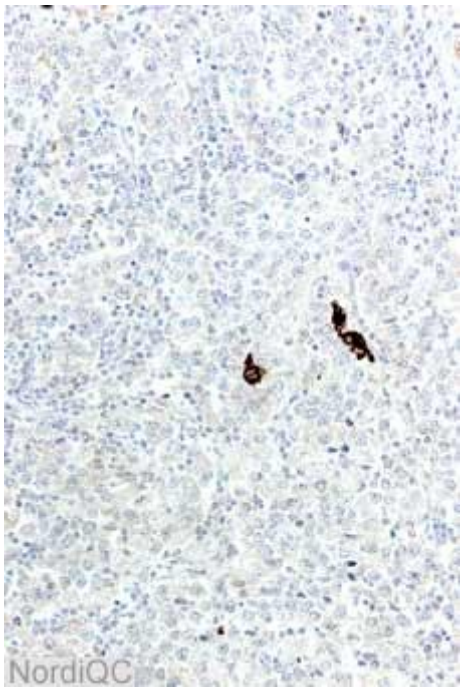


Fig. 2b
Optimal HCG staining of the seminoma. The trophoblast like cells are strongly stained while the neoplastic cells are weakly stained (same protocol as in Fig. 1b).

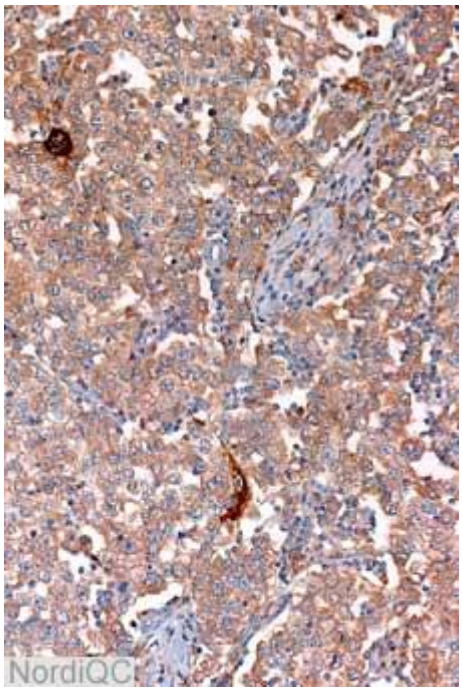


Fig. 2c
Insufficient HCG staining of the seminoma. The trophoblast like cells are strongly stained but a moderate staining of the neoplastic cells is seen as well (same protocol as in Fig. 1c).

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