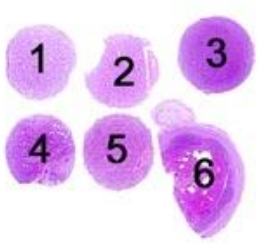
Assessment Run 24 2008

Octamer transcription factor-3/4 (OCT3/4)

The slide to be stained for Octamer transcription factor-3/4 (OCT3/4) 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 an OCT3/4 staining as optimal included:

- A strong and distinct nuclear reaction of the majority of the neoplastic cells of the two IGCNs, the seminoma and the embryonal carcinoma. A diffuse weak cytoplasmic reaction was accepted
- No reaction in the placenta and in the appendix.

18 laboratories submitted stains. At the assessment 12 (67%) laboratories achieved an optimal mark, 3 (17%) good and 3 (17%) marked as borderline.

The following Abs were used:
mAb clone **C-10** (Santa Cruz sc-5279, n=12; Zhongshan 2M-0233, n=1)
mAb clone **SEMGC** (Biocare PM313AA, n=2)
pAb (Santa Cruz sc-8629, n=3)

In this assessment an optimal staining for **OCT3/4** could only be obtained with the mAb clone **C-10**.

C10: the protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using an alkaline buffer as Tris-EDTA/EGTA pH 9 (7/8)*, Target Retrieval Solution, High pH (K8012, Dako; 2/2), Cell Conditioning1 (BenchMark, Ventana; 1/1), Bond Epitope Retrieval Solution 2 (Bond, Leica 1/1) and EDTA/EGTA pH 8 (1/1).
The mAb was typically diluted in the range of 1:40 – 1:1.000 depending on the total sensitivity of the protocol employed (pre-treatment equipments, heating incubation time, primary antibody incubation time and detection system). Using these protocol settings 13 out of 13 (100%) laboratories produced a sufficient staining (optimal or good) out of which 12 (93%) were optimal.
*number of optimal results/number of laboratories using this buffer

The most frequent causes of insufficient staining were:
- Less successful primary antibody
- Excessive background reaction

In this assessment the prevalent feature of an insufficient staining was a general too weak or false negative staining of the malignant cells in the IGCN, the embryonal carcinoma and the seminoma. When the polyclonal goat Ab from Santa Cruz (sc-8629) was used also an excessive background was seen. This background reaction might be caused by the choice and application of the detection system, which has to be raised toward goat immunoglobulin and in this assessment the laboratories used detection systems primarily designed for rabbit and mouse antibodies produced in goat.

At present no easily accessible normal tissue expressing OCT3/4 has been identified and IGCN seem to be the preferred recommendable control in which the neoplastic cells has to show an as strong as possible nuclear reaction with minimal cytoplasmic reaction, while other cells shall be negative.

It was noteworthy that the pass rate for OCT3/4 was 84% compared to the pass rate of 52% for PLAP. This should encourage laboratories to either add OCT3/4 to their panel of markers for germinal cell tumours or completely exchange PLAP with OCT3/4.

Conclusions
The mAb clone **C-10** was in this assessment a robust and suitable marker for OCT3/4. HIER in an alkaline buffer was mandatory to obtain an optimal staining. IGCN is recommended as a positive control in which the staining should be as strong as possible in the nuclei expected to stain – all other cells should be negative.

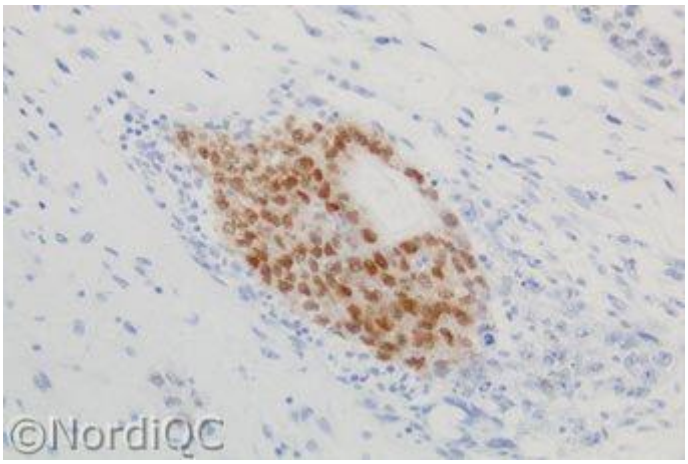


Fig. 1a
Optimal OCT3/4 staining of the embryonal carcinoma using the mAb clone C10 with HIER and optimally calibrated. The neoplastic cells show a distinct nuclear staining and only a weak cytoplasmic staining. The background is negative.

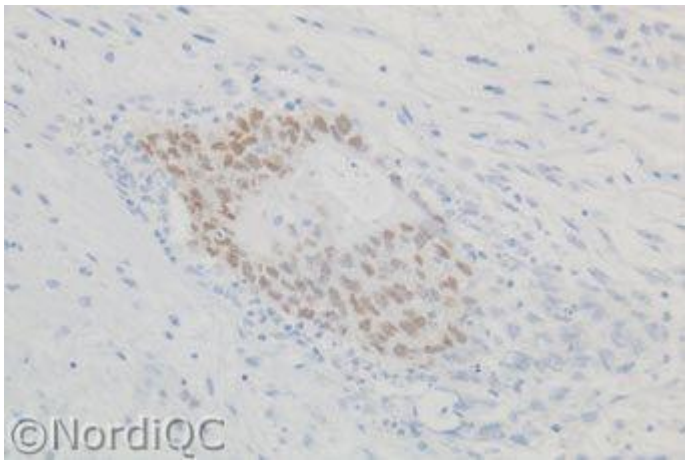


Fig. 1b
Insufficient OCT3/4 staining of the embryonal carcinoma using the mAb clone C10 with HIER, but too diluted – same field as in Fig. 1a. The neoplastic cells only show a weak and diffuse nuclear staining. Also compare with Fig. 2b – same protocol.

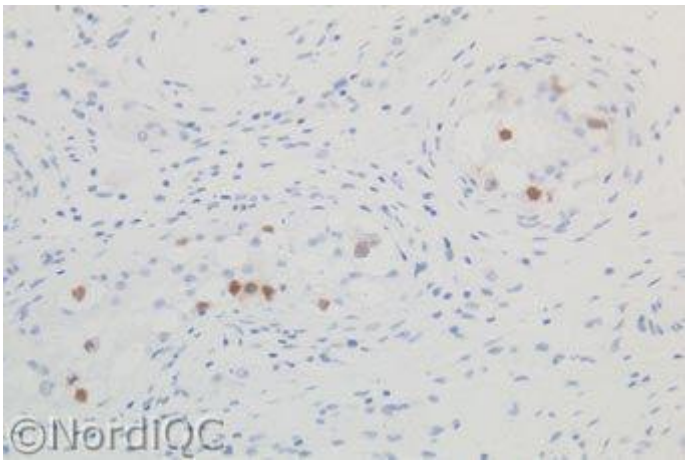


Fig. 2a
Optimal OCT3/4 staining of the intratubular germ cell neoplasia using same protocol as in Fig. 1a. The neoplastic cells show a strong distinct nuclear staining with no background reaction.

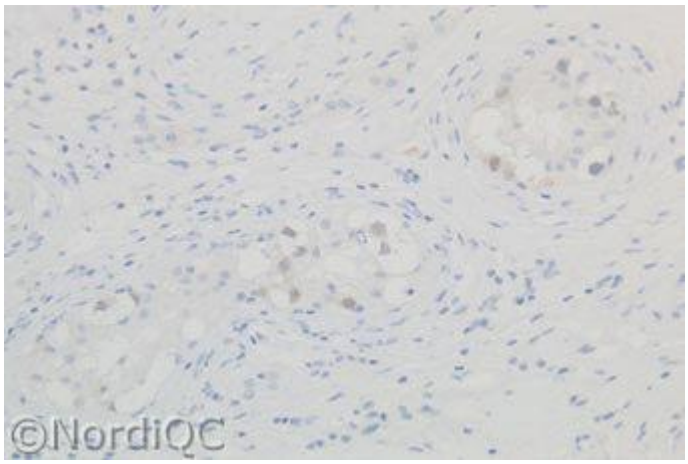


Fig. 2b
Insufficient OCT3/4 staining of the intratubular germ cell neoplasia using same protocol as in Fig. 1b. Only scattered neoplastic cells show a weak nuclear staining – same field as in Fig. 2a.

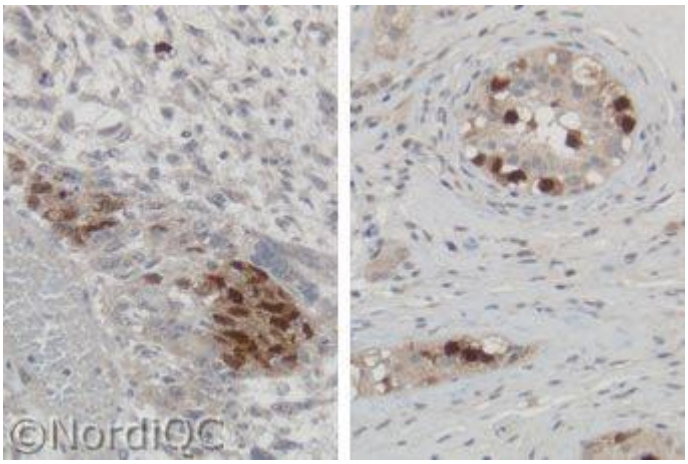


Fig. 3a
OCT3/4 staining using an insufficient protocol based on the goat polyclonal Ab sc-8629. Also compare with Fig. 3b. left – same protocol.
Left: The neoplastic cells of the embryonal carcinoma show a strong nuclear staining, but also a diffuse background reaction.
Right: The neoplastic cells of the intratubular germ cell neoplasia show a strong nuclear staining, but also a diffuse background reaction is seen.

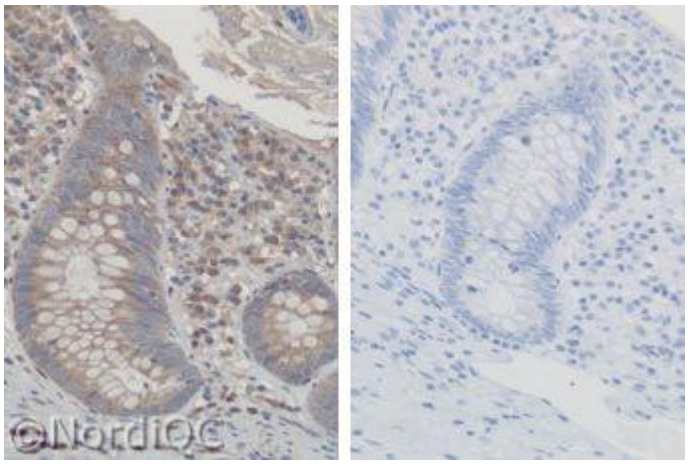


Fig. 3b
Appendix
Left: Insufficient staining using same protocol as in Fig. 3a. An excessive non-specific staining is seen in both the lymphocytes, epithelial and stromal cells.
Right: Optimal staining using same protocol as in Fig. 1a. and 2a. No staining is seen.

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