

Assessment Run 15 2005

Wilms' tumour-1 protein (WT1)

The slide to be stained for Wilm's tumour-1 protein (WT1) comprised:
 1. Fallopian tube, 2. Appendix, 3. Lung adenocarcinoma, 4. Mesothelioma,
 5. Ovarian serous carcinoma, moderately differentiated, 6. Granulosa cell tumour.
 All specimens were fixed in 10 % NBF.



Criteria for assessing a WT1 staining as optimal included:

- A strong and distinct nuclear staining in the epithelial cells of the fallopian tube.
- A strong and distinct nuclear staining of the ovarian serous carcinoma, the granulosa cell tumour and the mesothelioma.
- No nuclear staining in the epithelium of the appendix or the lung adenocarcinoma.

The cytoplasmic reaction in a variety of cells i.e. endothelial cells, smooth muscle cells and plasma cells is unavoidable and not included in the assessment.

24 laboratories submitted stains. At the assessment 4 achieved optimal marks (17 %), 5 good (21 %), 12 borderline (50 %) and 3 (12 %) poor marks.

The following Abs were used:
 mAb clone **6H-F2** (Dako, n=21; Novocastra, n=2)
 pAb **WT1 (C-19)** (Santa Cruz, n=1)

Optimal staining for WT1 in this assessment was obtained with the mAb clone **6H-F2** (4 out of 23).

All optimal protocols were based on heat induced epitope retrieval (HIER) in Tris-EDTA/EGTA pH 9 (4 out of 14). The clone **6H-F2** was used in the range of 1:25– 1:400 depending on the sensitivity of the protocol applied. Using proteolytic pre-treatment in pepsin as recommended by Dako (data sheet) could not be used to obtain an optimal staining.

The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Insufficient or inappropriate epitope retrieval (too short HIER in citrate pH 6, or proteolytic pre-treatment).

In the interpretation only a nuclear reaction was considered as a true positive reaction of WT1. In general most laboratories were capable to demonstrate WT1 in the serous ovarian carcinoma and the mesothelioma. The prevalent feature of an insufficient staining was typically characterized by a too weak or a false negative reaction of the granulosa cell tumour.

Almost all laboratories could demonstrate WT1 in the fallopian epithelium, which indicates that this is not an optimal control. Using fallopian tube, the epithelial and smooth muscle cells should show a strong nuclear staining with only a minimal cytoplasmic reaction.

Conclusion

mAb **6H-F2** seems to be a good marker for WT1. HIER in Tris-EDTA/EGTA pH 9 seems to be the best pre-treatment.

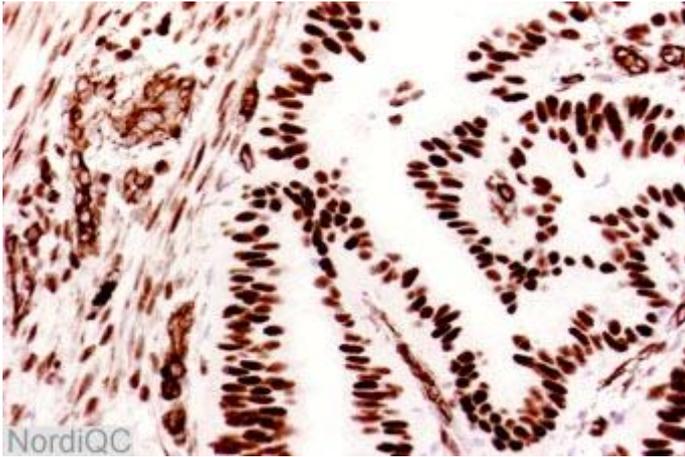


Fig. 1a
Optimal staining for WT1 in the fallopian tube. All the epithelial cells and smooth muscle cells show a strong distinct nuclear staining.

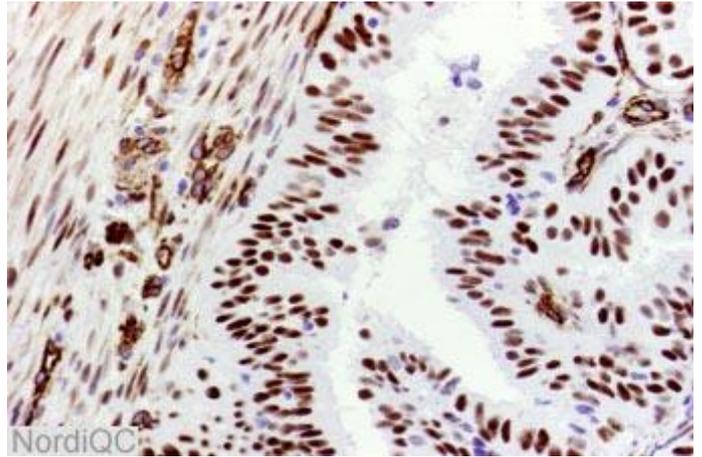


Fig. 1b
Staining for WT1 in the fallopian tube using an insufficient protocol (same field as in Fig. 1a.). The majority of the epithelial cells and smooth muscle cells show a distinct nuclear staining. However compare with Fig. 2b and Fig. 3b – same protocol.

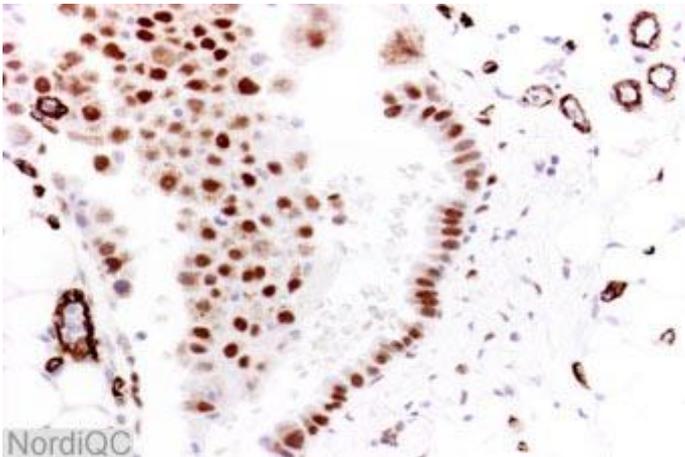


Fig. 2a
Optimal staining for WT1 of the mesothelioma. Both the normal and the neoplastic mesothelial cells show a strong distinct nuclear staining.

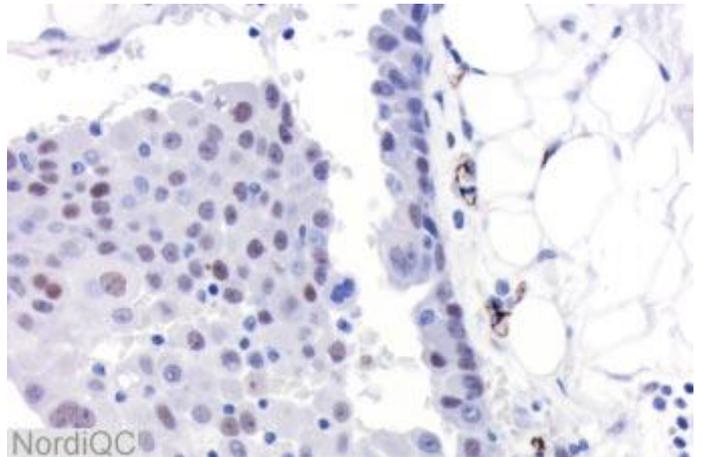


Fig. 2b
Insufficient staining for WT1 of the mesothelioma. Both the normal and the neoplastic mesothelial cells are unstained or only show a weak nuclear staining. (same protocol used in Fig. 1b).

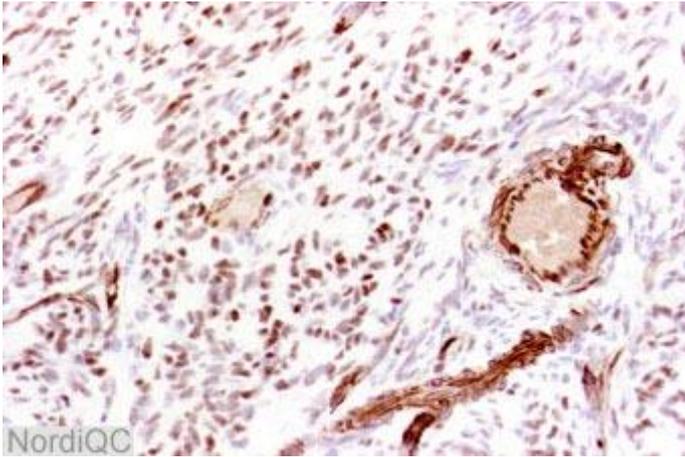


Fig. 3a
Optimal staining for WT1 of the granulosa cell tumour. The majority of the neoplastic cells show a distinct nuclear staining. The endothelial cells show a cytoplasmic staining.

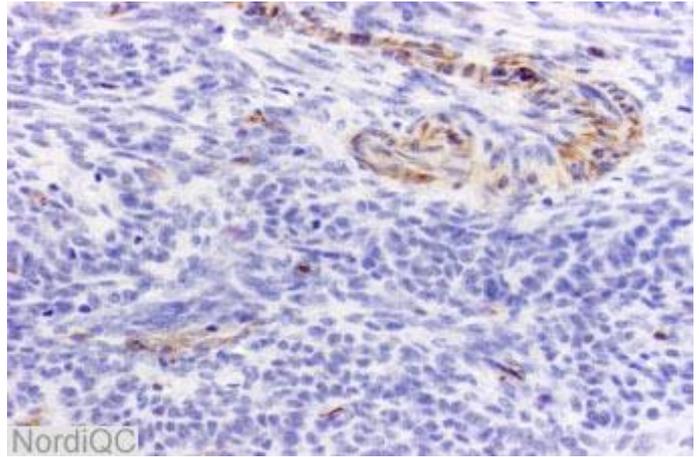


Fig. 3b
Insufficient staining for WT1 of the granulosa cell tumour. The majority of the neoplastic cells are unstained or only weakly positive (same protocol used in Fig. 1b and 2b). The endothelial cells show a cytoplasmic staining.

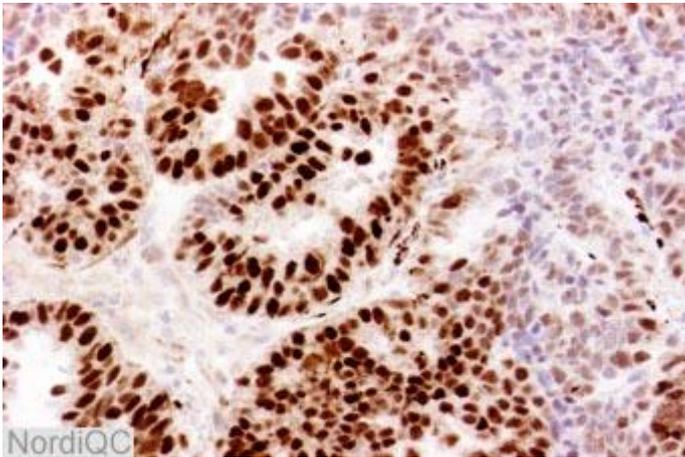


Fig. 4a
Optimal staining for WT1 of the ovarian serous carcinoma. The majority of the neoplastic cells show a distinct nuclear staining.

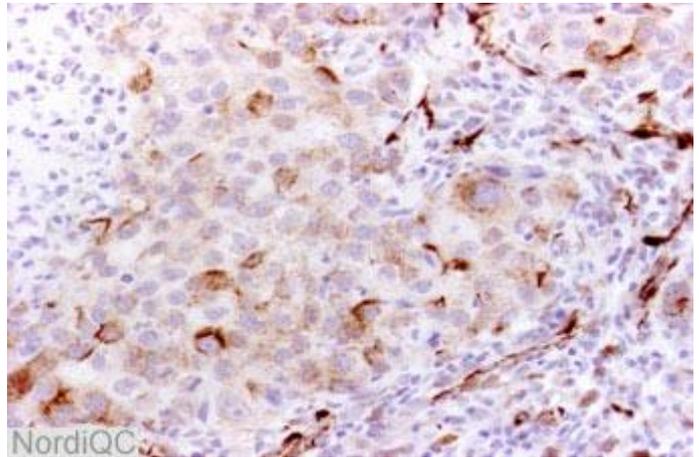


Fig. 4b
Staining for WT1 of the lung adenocarcinoma. The neoplastic cells show a non-specific cytoplasmic staining (which is acceptable) but no nuclear staining. Same protocol used in Fig. 4a.

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