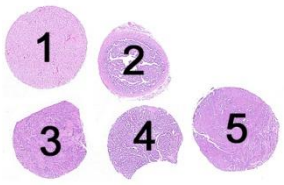
Assessment Run 28 2010

Wilms' tumour-1 protein (WT1)

The slide to be stained for WT1 comprised:
1. Kidney, 2. Fallopian tube, 3. Lung adenocarcinoma, 4. Ovarian serous carcinoma, 5. Uterine endometrioid carcinoma.
All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a WT1 staining as optimal included:

- A strong and distinct nuclear staining in virtually all the epithelial and smooth muscle cells of the fallopian tube.
- A strong and distinct nuclear staining in virtually all the neoplastic cells of the ovarian serous carcinoma.
- A moderate to strong nuclear staining in the stromal cells of the uterine endometrioid carcinoma.
- No staining of the renal tubules or the lung adenocarcinoma.

A cytoplasmic reaction in a variety of cells, e.g., endothelial cells, smooth muscle cells and plasma cells was expected and accepted for the mAb clone 6F-H2.

96 laboratories participated in this assessment. 83 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for WT1, run 28**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **6H-F2**	61 3 1	Dako Cell Marque Master Diagnostica	26	26	4	9	80 %	81 %
mAb clone **WT49**	12 1	Novocastra Monosan	6	5	2	0	85 %	89 %
pAb **RB-9267**	2	NeoMarkers	0	2	0	0	-	-
pAb **C-19**	1	Santa Cruz	0	0	1	0	-	-
Ready-To-Use Abs								
mAb clone **6H-F2, IR055**	7	Dako	5	2	0	0	100 %	100 %
mAb clone **6H-F2, 760-4397**	5	Ventana / Cell Marque	1	3	1	0	80 %	-
mAb clone **WT49, PA0562**	2	Leica	1	1	0	0	-	-
mAb clone **BC.6H-F2, PM258**	1	Biocare	0	1	0	0	-	-
Total	96		39	40	8	9	-	-
Proportion			41 %	42 %	8 %	9 %	83 %	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **6H-F2**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using one of the following buffers: Tris-EDTA/EGTA pH 9 (12/19)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (8/11), Target Retrieval Solution pH 9.9 (S3307, Dako) (1/1), Cell Conditioning 1 (BenchMark, Ventana) (1/20), Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/3), Nuclear Decloaker pH 9,5 (Biocare) (1/1), EDTA/EGTA pH 8 (2/2)* or Citrate pH 6 (1/3). The mAb was typically diluted in the range of 1:30– 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 44 out of 54 (81 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **WT49**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using one of the following buffers: Tris-EDTA/EGTA pH 9 (3/5), Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (1/1), or Bond Epitope Retrieval Solution 2 (Bond, Leica) (2/3). The mAb was typically diluted in the range of 1:10– 1:60 depending on the total sensitivity of the protocol employed. Using these protocol settings 8 out of 9 (89 %) laboratories produced a sufficient staining.

Ready-To-Use Abs
mAb clone **6H-F2** (prod. no IR055, Dako): The protocols giving an optimal result were all based on HIER in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings all of 7 (100 %) laboratories produced a sufficient staining.

mAb clone **6H-F2** (prod. no. 760-4397, Ventana / Cell Marque): The protocol giving an optimal result was based on HIER using standard Cell Conditioning 1 (BenchMark, Ventana), an incubation time of 32 min in the primary Ab and Ultra View (760-500) with amplification as the detection system. Using these protocol settings both of 2 laboratories produced a sufficient staining.

mAb clone **WT49** (prod. no. PA0562, Leica), the protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 1 (Bond, Leica), an incubation time of 15 min in the primary Ab and BOND Polymer Refine Detection (DS9800) as the detection system. Using these protocol settings both of 2 laboratories produced a sufficient staining.

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Insufficient epitope retrieval (too short efficient HIER).

In this assessment the prevalent feature of an insufficient staining was a too diffuse or completely false negative nuclear staining reaction of the cells expected to be demonstrated. This pattern was seen in both the normal epithelial cells of the fallopian tube and the neoplastic cells of the ovarian serous carcinoma.
The newly launched mAb clone WT49 gave a reaction pattern different from mAb clone 6F-H2. In an optimally calibrated protocol based on the mAb clone WT49 with HIER, a distinct nuclear staining was obtained in the epithelial cells of the fallopian tube and in the serous carcinoma, whereas no cytoplasmic reaction was seen in the endothelial cells and muscle cells. Also a distinct nuclear reaction was seen in the parietal epithelial cells of the Bowman capsule and the podocytes in the renal glomeruli. With mAb clone 6F-H2 in an optimally calibrated protocol based on HIER, the parietal epithelial cells and podocytes also show a nuclear staining, but this reaction is difficult to interpret due to an extensive cytoplasmic reaction of the endothelial cells. When the mAb clone 6F-H2 was used with proteolytic pre-treatment, no cytoplasmic reaction in the endothelial cells and muscle cells was seen. However, in this assessment no optimal staining reaction was obtained, when proteolytic pre-treatment was used, primarily due to a too weak intensity and reduced proportion of positive cells. Noteworthy, the recommended protocol from Dako (the most used vendor) for the mAb clone 6F-H2 as a concentrate is based on proteolytic pre-treatment, whereas HIER is recommended when the clone is sold as a Ready-To-Use (RTU) format from same vendor.

The preferred control for WT1 seems to be fallopian tube in which the epithelial cells must show an as strong as possible nuclear reaction with only a minimal cytoplasmic reaction. Kidney, especially when using the mAb clone WT49, can also be recommended: At least a moderate nuclear staining must be seen in the parietal epithelial cells and podocytes of the Bowman capsule, while the epithelial cells of the tubules should show no nuclear or cytoplasmic staining.

This was the second assessment of WT1 in NordiQC. The proportion of sufficient results showed a significant increase from 38 % in run 15, 2005 to 83 % in the current run – see table 2. The higher pass rate may be due many factors including the availability of new improved clones and RTU formats for WT1 Abs.

Table 2. **Proportion of sufficient results for WT1 in the two NordiQC runs performed**

	Run 15 2005	Run 28 2010
Participants, n=	24	96
Sufficient results	38 %	83 %

Conclusion
The mAb clones 6F-H2 and WT49 are recommendable clones for WT1. For both clones HIER seems mandatory to obtain an optimal staining. However HIER will induce a cytoplasmic reaction for the mAb clone 6F-H2

Fallopian tube is an appropriate control for WT1: Virtually all the epithelial cells and the smooth muscle cells shall show an as strong as possible nuclear reaction with only a minimal cytoplasmic reaction.

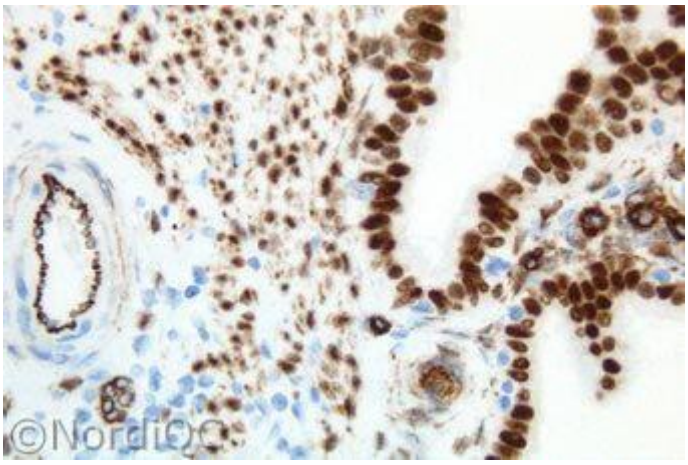


Fig. 1a
Optimal WT1 staining of the fallopian tube using mAb clone 6F-H2 optimally calibrated and with HIER in an alkaline buffer. Virtually all the epithelial cells and the majority of the stromal cells show a strong and distinct nuclear staining. A strong cytoplasmic staining is seen in the endothelial cells, while no background staining is seen.

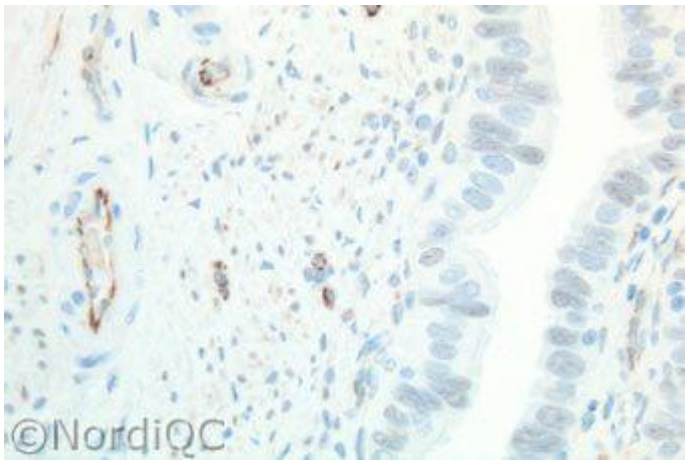


Fig. 1b
Insufficient WT1 staining of the fallopian tube using the mAb clone 6F-H2 too diluted. The epithelial and the stromal cells only show a weak and diffuse nuclear staining, while the endothelial cells show a moderate cytoplasmic staining – also compare with Figs. 2b – 3b, same protocol.

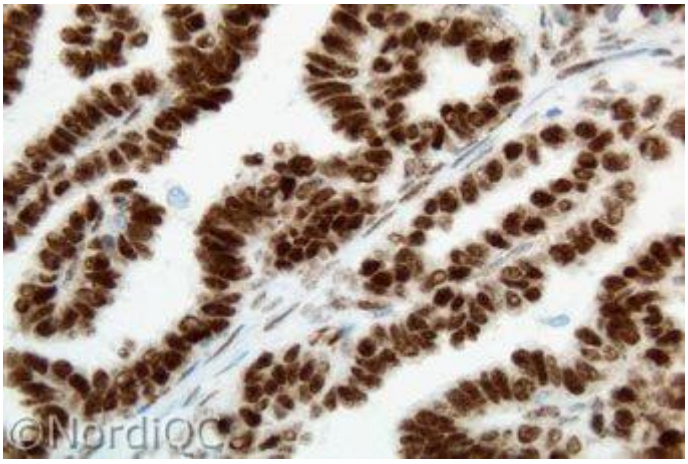


Fig. 2a
Optimal WT1 staining of the ovarian serous carcinoma using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a strong and distinct nuclear staining.

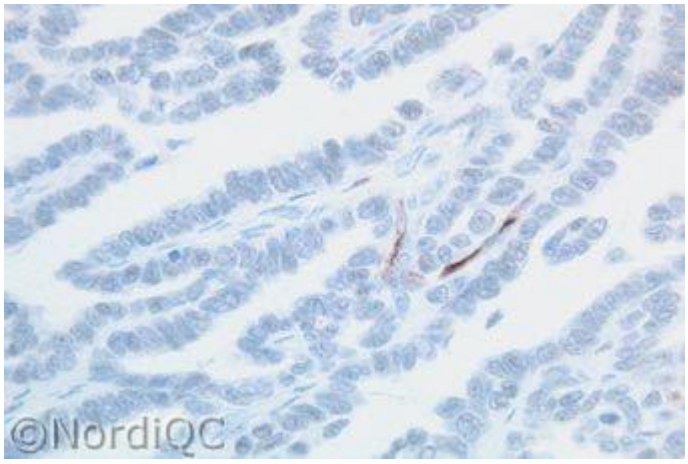


Fig. 2b
Insufficient WT1 staining of the ovarian serous carcinoma using same protocol as in Fig. 1b. The neoplastic cells are virtually negative and only the endothelial cells show a cytoplasmic staining.

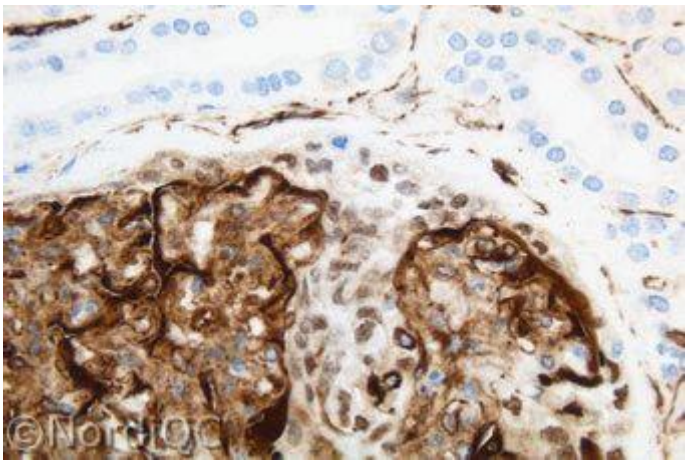


Fig. 3a
Optimal WT1 staining of the kidney using the same protocol as in Figs. 1a & 2a. The podocytes and parietal epithelial cells show a moderate, distinct nuclear staining. No staining is seen in the tubules.

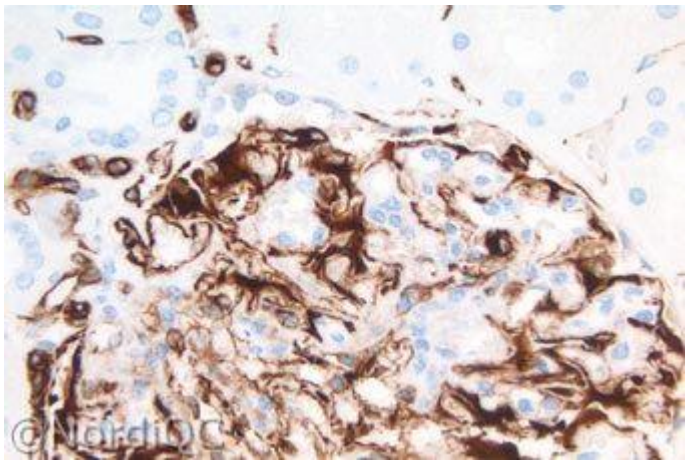


Fig. 3b
Insufficient WT1 staining of the kidney using same protocol as in Figs. 1b & 2b. Only the endothelial cells in the glomeruli show a cytoplasmic staining, while the podocytes and parietal epithelial cells show no nuclear staining.

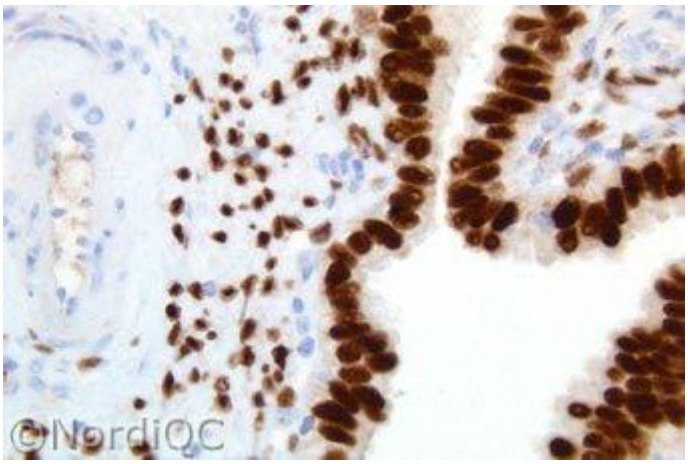


Fig. 4a
Optimal WT1 staining for of the fallopian tube using the mAb clone WT49 optimally calibrated and with HIER in an alkaline buffer. Virtually all the epithelial cells and the majority of the stromal cells show a strong and distinct nuclear staining. No cytoplasmic staining is seen in the endothelial cells (compare with Fig. 1a, where the mAb clone 6F-H2 is used with similar protocol settings).

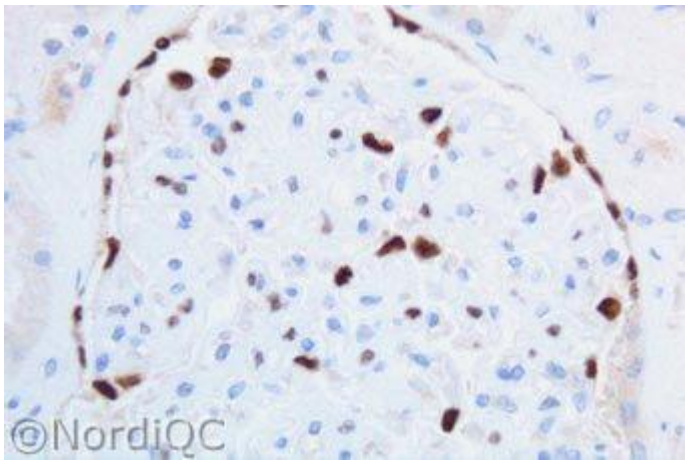


Fig. 4b
Optimal WT1 staining for WT1 of the kidney using same protocol as in Fig. 4a. The podocytes and parietal epithelial cells show a moderate to strong distinct nuclear staining. No cytoplasmic staining is seen in the endothelial cells (compare with Fig. 3a, where the mAb clone 6F-H2 is used with similar protocol settings).

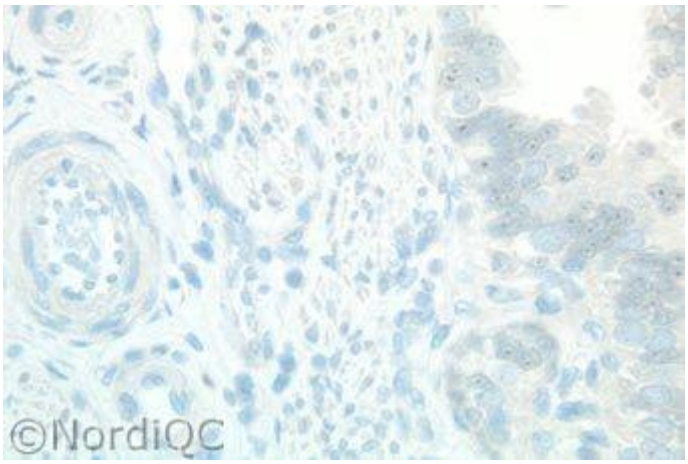


Fig. 5a
Insufficient WT1 staining of the fallopian tube using the mAb clone 6F-H2 with proteolytic pre-treatment. The epithelial cells and stromal cells are virtually negative and show no specific nuclear staining. The endothelial cells show no cytoplasmic staining. Also compare with Fig. 5b, same protocol.

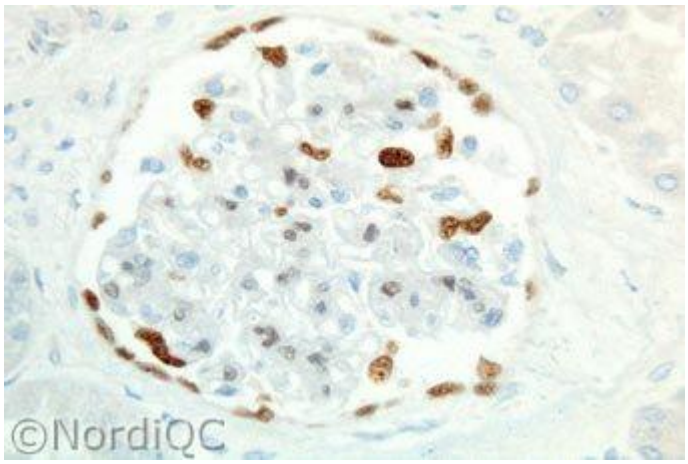


Fig. 5b
WT1 staining of the kidney using same insufficient protocol as in Fig. 5a.
A distinct nuclear staining is seen in the podocytes and the parietal epithelial cells, while no cytoplasmic staining is seen in the endothelial cells. This reaction pattern was typically seen when the mAb clone 6F-H2 was used with proteolytic pre-treatment. Also compare with Figs. 3a and 3b – mAb clone 6F-H2 with HIER.

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