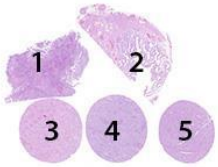
Assessment Run 55 2019

Wilms' tumour-1 protein (WT1)

Material
The slide to be stained for WT1 comprised:

1. Mesothelioma, 2. Fallopian tube, 3. Kidney, 4. Lung adenocarcinoma,
5. Serous ovarian 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 of virtually all epithelial and smooth muscle cells in the fallopian tube
- A strong and distinct nuclear staining of virtually all neoplastic cells in the ovarian serous carcinoma
- A strong and distinct nuclear staining of virtually all neoplastic cells in the mesothelioma
- A moderate to strong nuclear staining of podocytes and parietal epithelial cells in kidney
- No staining of the lung adenocarcinoma or the renal tubules

A cytoplasmic staining reaction in a variety of cells, e.g. endothelial, smooth muscle, striated muscle and plasma cells, was expected and accepted for the mAb clone 6F-H2. In the majority of cells, a distinct dot-like staining of nucleoli was seen and accepted for the rmAb clone EP122.

Participation

Number of laboratories registered for WT1, run 55	298
Number of laboratories returning slides	291 (98%)

Results
291 laboratories participated in this assessment. 263 (90%) achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Less successful performance of mouse monoclonal Ab (mAb) clone 6F-H2 on the Ventana BenchMark platform
- Use of less sensitive detection systems
- Too low concentration of the primary antibody
- Insufficient Heat Induced Epitope Retrieval (HIER) – too short efficient heating time

Performance history
This was the fourth NordiQC assessment of WT1. An increase in the pass rate was observed compared to the latest run (Run 43 in 2015) as listed in Table 2.

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

	Run 15 2005	Run 28 2010	Run 43 2015	Run 55 2019
Participants, n=	24	96	220	291
Sufficient results	38%	83%	81%	91%

Conclusion
The mAb clones **6F-H2**, **WT49** and the rmAb clones **EP122** and **D8I7F** are all recommendable antibodies for demonstrating WT1. Irrespective of the clone applied, efficient HIER (preferable in an alkaline buffer, either alone or in combination with short protease treatment), careful calibration of the primary Ab and use of a 3-step multimer/polymer-based detection system, were the main prerequisites for an optimal result. A combined HIER–protease retrieval protocol was used by many laboratories (n=45) in this WT1 assessment. On the Ventana BenchMark platform this retrieval protocol provided a significant improvement in the signal-to-noise ratio for the mAb clone 6F-H2 (both as concentrate and Ready-To-Use (RTU) system).

The RTU systems for WT1 from Dako (clone 6F-H2 based) and Leica (clone WT49 based) provided the highest proportion of sufficient and optimal staining results. Both systems produced a proportion of

sufficient staining results of 100%. Following the recommended protocol settings, the RTU system from Ventana (clone 6F-H2 based) was less successful, but modified protocol settings (implementing combined retrieval and the use of OptiView detection system) led to improvements, resulting in a proportion of sufficient staining results of 96%.

Depending on the clone and retrieval procedures, both fallopian tube and kidney can be used as positive tissue controls for WT1. In fallopian tube, a strong, distinct nuclear staining reaction of the majority of the epithelial cells and smooth muscle cells must be seen. Kidney can serve as both positive and negative tissue control: Podocytes and the parietal epithelial cells of Bowman's capsule should display a strong, distinct nuclear staining, whereas epithelial cells of the tubules should be negative.

Table 1. **Antibodies and assessment marks for WT1, Run 55**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **6F-H2**	52 13 2 2 2 2 1	Dako/Agilent Cell Marque BioCare DCS Diagnostic BioSystems Immunologic Zeta	36	31	6	1	91%	92%
mAb clone **WT49**	13 1	Leica Immunologic	11	2	0	1	93%	100%
rmAb clone **D8I7F**	3	Cell Signaling	3	0	0	0	-	-
rmAb clone **EP122**	3 1	Epitomics Cell Marque	3	1	0	0	-	-
pAb **RB-9367-P**	1	Neomarkers	0	0	1	0	-	-
Ready-To-Use Antibodies								
mAb clone **6F-H2 760-4397**	92	Ventana/Cell Marque	40	37	14	1	84%	94%
mAb clone **6F-H2 IR055/IS055**	33	Dako/Agilent	30	3	0	0	100%	100%
mAb clone **6F-H2 IR055/IS055** [3]	25	Dako/Agilent	21	3	1	0	96%	-
mAb clone **6F-H2 IR055/IS055** [4]	9	Dako/Agilent	5	3	1	0	-	-
mAb clone **6F-H2 348M-98** [5]	14	Cell Marque	5	7	2	0	86%	-
mAb clone **6F-H2 MAD-005671QD**	2	Master Diagnostica	2	0	0	0	-	-
mAb clone **MX012 MAB-0678**	1	Maixin	1	0	0	0	-	-
mAb clone **WT49 PA0562**	17	Leica	17	0	0	0	100%	100%
mAb clone **WT49 PA0562** [6]	1	Leica	1	0	0	0	-	-
rmAb clone **EP122 8340**	1	Sakura	1	0	0	0	-	-
Total	291		176	87	25	3	-	
Proportion			60%	30%	9%	1%	90%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on the Dako/Agilent full-automatic platform (Dako Omins)
4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on different platforms (e.g. Ventana Benchmark, BioCare IntelliPath and Leica Bond).
5) RTU format not developed for a specific platform, but used by laboratories on the Ventana Benchmark platform.
6) RTU system developed for the Leica Bond system, but used on the Ventana Benchmark platform.

Detailed analysis of WT1, Run 55
The following protocol parameters were central to obtain optimal staining:

Concentrated Antibodies
mAb clone **6F-H2**: Protocols with optimal results were based on HIER using either Cell Conditioning 1 (CC1, Ventana) (10/24)*, Bond Epitope Retrieval Solution 2 (BERS2, Leica) (8/13), Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9, Dako) (8/9), Target Retrieval Solution, High pH (TRS pH 9, Dako), Target Retrieval Solution, low pH (3-in-1) (TRS pH 6.1, Dako) (1/1) Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/2), Tris-EDTA/EGTA pH 9 (1/1) or DBS Montage EDTA (Diagnostic BioSystems) (1/1) as retrieval buffer. Optimal results were also obtained using a combination of HIER in CC1 (Ventana) and Protease 3 pretreatment (Ventana) (4/12). The mAb was typically diluted in the range of 1:25-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 61 of 66 (92%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **WT49**: Protocols with optimal results were all based on HIER using either CC1 (Ventana) (4/5), BERS2 (Leica) (3/4), TRS pH 9 (3-in-1) (Dako) (3/4) or TRS pH 9 (Dako) 1/1). The mAb was typically diluted in the range of 1:5-1:25 depending on the total sensitivity of the protocol employed. Using these protocol settings, 12 of 12 (100%) laboratories produced a sufficient staining result.

rmAb clone **D8I7F**: Protocols with optimal results were all based on HIER using either TRS pH 9, (Dako) (2/2) or CC1 (Ventana) (1/1). The rmAb was diluted in the range of 1:45-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 3 of 3 (100%) laboratories produced an optimal staining result.

rmAb clone **EP122**: Protocols with optimal results were all based on HIER using either CC1 (Ventana) (1/1), TRS pH 9 (Dako) (1/1) or Tissue-Tek Genie High pH Antigen Retrieval Solution (Sakura) (1/1). The rmAb was typically diluted in the range of 1:25-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 3 of 3 (100%) laboratories produced an optimal staining result.

Table 3. **Proportion of optimal results for WT1 for the most commonly used antibodies as concentrates on the four main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**			**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC1 pH 8.5 + Protease 3	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **6F-H2**	8/9** 89%	1/1	2/6 33%	-	10/24 42%	4/12 33%	-	8/13 62%	1/2
mAb clone **WT49**	2/3	-	1/1	-	4/5 80%	-	-	3/4	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** Number of optimal results/number of laboratories using this buffer

Ready-To-Use (RTU) Antibodies and corresponding systems
mAb clone **6F-H2** product no. **760-4397**, Ventana/Cell Marque, BenchMark GX, XT and Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min.) or CC1 (efficient heating time 16-32 min.) followed by weak proteolysis, typically in Protease 3 for 4-8 min., 8-32 min. incubation of the primary Ab and OptiView (760-700) or OptiView with tyramide amplification (760-099/860-099) as detection system. Using these protocol settings, 62 of 66 (94%) laboratories produced a sufficient staining.

mAb clone **6F-H2** product no. **IR055/IS055**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 10-30 min. at 95-100°C), 15-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002/GV800) as detection system. Using these protocol settings, 32 of 32 (100%) laboratories produced a sufficient staining result.

mAb clone **6F-H2**, product no. **MAD-005671QD-7/N**, Master Diagnostica, LabVision:
Protocols with optimal results were based on HIER using EDTA/EGTA pH 8, efficient heating time 20 min. at 95-97°C, 10 min. incubation of the primary Ab and Master Polymer Plus (MAD-000237QK/N) as detection system. Using these protocol settings, 2 of 2 (100%) laboratories produced an optimal staining result.

mAb clone **WT49** product no. **PA0562**, Leica/Novocastra, Leica Bond-III/Bond-Max:
Protocols with optimal results were typically based on HIER using BERS2 pH 9 (efficient heating time 20-40 min. at 99-100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 17 of 17 (100%) laboratories produced an optimal staining result.

mAb clone **MX012**, product no. **MAB-0678**, Maixin, manual:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (Waterbath) for 20 min., 60 min. incubation of the primary Ab and MaxVision III DAB (Maixin) as detection system.

rmAb clone **EP122,** product no. **8340**, Sakura Finetek, Genie:
One protocol with an optimal result was based on 30 min. HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer, 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for WT1 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone **6F-H2, 760-4397**	80% (20/25)	20% (5/25)	85% (57/67)	52% (35/67)
Dako AS mAb clone **6F-H2, IR055/IS055**	100% (21/21)	95% (20/21)	100% (12/12)	83% (10/12)
Leica Bond mAb clone **WT49, PA0562**	100% (8/8)	100% (8/8)	100% (9/9)	100% (9/9)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments
In concordance with the previous assessments for WT1 in NordiQC, the prevalent feature of insufficient staining results was a too weak or completely false negative nuclear staining reaction of cells expected to be demonstrated. This was observed in 79% of the insufficient results (22 of 28). The remaining 6 cases (21%) showed an overall poor signal-to-noise ratio due to excessive background staining often in combination with too weak specific staining.

The majority of the participating laboratories were able to demonstrate WT1 in epithelial cells of the fallopian tube, podocytes and parietal epithelial cells in kidney, whereas demonstration of WT1 in neoplastic cells of the mesothelioma, ovarian serous carcinoma and in smooth muscle cells of the fallopian tube was more challenging and only seen with appropriate protocol settings (Fig. 1a to Fig. 4b). Both fallopian tube and kidney can be used as positive tissue controls, but if available, fallopian tube should be preferred, as smooth muscle cells of the fallopian tube seem to express lower levels of WT1 than both podocytes and parietal epithelial cells in kidney.

33% (96 of 291) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for WT1. Optimal demonstration of WT1 could be achieved with the mAb clones 6F-H2, WT49 and the rmAb clones D8I7F and EP122 (see Table 1). The mAb clones 6F-H2 and WT49 were the most widely used Abs and both could produce optimal staining on all the main stainer platforms (see Table 3). In optimally calibrated HIER-based protocols, the mAb WT49 gave a strong and exclusively nuclear staining reaction in epithelial and smooth muscle cells of the fallopian tube, neoplastic cells of the ovarian serous carcinoma, neoplastic cells of the mesothelioma and in podocytes and parietal epithelial cells in kidney. With mAb clone 6F-H2 in an optimally calibrated protocol based on HIER, strong nuclear staining was seen in all the expected cells. In addition to the nuclear staining reaction, clone 6F-H2 also provided cytoplasmic staining reaction in plasma cells, smooth muscle cells and endothelial cells. This staining pattern was expected and accepted, but it complicates the interpretation e.g. the nuclear staining in the podocytes and parietal epithelial cells of the Bowman capsule is hard to recognize due to a strong cytoplasmic reaction (Fig. 2a, Fig. 2b and Fig. 7a). The recommended protocol (from the majority of vendors) for the mAb clone 6F-H2 as a concentrate is based on HIER in an alkaline buffer, but in the previous assessment (Run 43,

2015), combination of HIER at high pH and short/weak proteolytic pretreatment was introduced for this clone. In Run 43 a total (concentrates and RTUs) of 9 laboratories used this combination. In the present run, this number has increased to a total of 45 laboratories. In the optimally calibrated protocols, combined HIER and weak proteolysis results in a staining pattern showing a strong nuclear positive staining reaction virtually without any cytoplasmic staining reaction in endothelial and smooth muscle cells (Fig. 5b and Fig. 7b). The most recently introduced WT1 Abs, the rmAb clones D8I7F and EP122, both performed very well. 3 laboratories using rmAb clone D8I7F on the Ventana BenchMark (n=1) or Dako Omnis (n=2) platforms were all assessed as optimal, giving a strong and exclusively nuclear staining reaction in epithelial and smooth muscle cells of the fallopian tube, neoplastic cells of the ovarian serous carcinoma, neoplastic cells of the mesothelioma and in podocytes and parietal epithelial cells in kidney. Similar results were seen in 4 laboratories using rmAb clone EP122 on the Ventana BenchMark (n=1), Dako Omnis (n=1) and Sakura Genie (n=2) platforms. The staining pattern was similar to the pattern seen with the clones D8I7F and WT49 with one exception. On the Sakura Genie platform an additional moderate to strong, distinct staining of the nucleoli in the majority of cells were displayed (Fig. 9b). The nature of this aberrant staining reaction is unknown, but as it did not interfere with the interpretation of the specific nuclear WT1 reaction, no downgrading was done on that basis. On the Ventana BenchMark and Dako Omnis platforms this aberrant staining reaction was either absent or only very faint (Fig. 9a). This platform related difference may be related to differences in washing temperatures. Ventana BenchMark and Dako Omnis stainers both rely on very efficient washing procedures performed at elevated temperatures; 36°C and 32°C respectively, whereas the washing procedure is performed at room temperature on the Sakura Genie stainer.

Optimal staining results could be achieved on the four main staining platforms, when the mAb clones 6F-H2 and WT49 were used as concentrates (see Table 3). For both clones, careful calibration of the titre of the primary Ab, efficient HIER in alkaline buffer, alone or in combination with weak proteolysis and use of a 3-step polymer/multimer detection system were the main protocol prerequisites for optimal results. Although high and similar proportions of sufficient staining results were achieved on all platforms with these clones, the proportion of optimal staining results for mAb clone 6F-H2 was significantly lower on the Ventana BenchMark platform compared to the Dako Autostainer and Leica Bond platforms (see Table 3). In the present run, 39% (14 of 36) of all laboratories using clone 6F-H2 on the Ventana platform were assessed as optimal, whereas the numbers for the Dako Autostainer and Leica Bond platforms were 89% (8 of 9) and 62% (8 of 13), respectively. Similar low proportion of optimal staining results was seen on the Dako Omnis platform, indicating that optimizing 6F-H2 protocols is challenging on these two platforms. In spite of this, comparison of data from the present assessment with Run 43 (2015) shows significant improvement for the Ventana BenchMark 6F-H2 LD-assays. In Run 43, the proportion of sufficient and optimal staining results was 67% (28 of 42) and 12% (5 of 42), respectively. In the present assessment these proportions have improved to 92% (33 of 36) and 39% (14 of 36), respectively. The main reason for this improvement was an increased use of 3-step multimer detection system (e.g. OptiView, with or without amplification). In Run 43, only 36% (15 of 42) of the laboratories used a 3-step multimer detection system, while this increased to 92% (33 of 36) in the present run.

Although the number of participants using the rmAb clone D8I7F within a LD assay was low, this primary Ab seems robust and promising, as all staining reactions (3 of 3) were assessed as optimal (see Table 1), showing strong specific nuclear staining reactions without any background staining on both the Ventana BenchMark and Dako Omnis platforms (Fig. 6a and Fig. 8a).

RTU antibodies was used in 67% (195 of 291) of the laboratories. The RTU systems based on mAb clone WT49 (Leica, PA0562) intended for the Bond platform and the mAb clone 6F-H2 based system (Dako, IR055/IS055) intended for the Dako Autostainer platform both performed better than protocols based on the corresponding concentrates. For both systems, the proportion of sufficient staining results was 100% irrespectively of recommended protocol settings (given by the vendor) or laboratory modified protocol settings was followed (see Table 4). The IR/IS0562 RTU, developed for the semi-automated Dako Autostainer platform, was used by many laboratories (n=25) on the fully-automated Dako Omnis platform. The proportion of sufficient staining results reached 96% (24 of 25) with 84% (21 of 25) optimal. The most widely used RTU system was the mAb clone 6F-H2 based (760-4397) system from Ventana, intended for use on the Ventana BenchMark platform. In contrast to the other RTU systems, 760-4397 had a lower proportion of sufficient staining results compared to the corresponding concentrate. 92 laboratories used the 760-4397, but only 27% (25 of 92) followed the recommended protocol settings given by Ventana. Using the recommended protocol settings resulted in a pass rate (sufficient staining result) of 80% with 20% being optimal. Two different protocol settings were given by Ventana. One protocol was based on the use of a 2-step multimer detection system (UltraView), the other was based on a 3-step multimer detection system (OptiView). The OptiView protocol recommendations were clearly the most successful of the two. The OptiView protocol had a pass rate of 91% (10 of 11) with 36% (4 of 11) optimal. In comparison, the UltraView had a pass rate of 71% (10 of 14) with only 7% (1 of 14) optimal. 73% (67 of

92) of the laboratories used the 760-4397 in laboratory modified protocol settings, resulting in both higher pass rate and proportion of optimal staining results. The most successful modifications were based on combined retrieval (HIER in CC1 combined with weak proteolysis in protease) and the use of a 3-step multimer detection system (e.g. OptiView, with or without amplification). This type of modification produced a pass rate of 96% (28 of 29) with 66% (19 of 29) optimal.

This was the fourth NordiQC assessment of WT1. In spite of many new laboratories participating, an increase in the pass rate was observed compared to the latest run (Run 43 in 2015) as listed in Table 2. The pass rate for new participants was 84% (95 of 113) compared to 94% (168 of 178) for laboratories participating more than once in a WT1 assessment. Some of the improvement in pass rate might be explained by the increased use of 3-step polymer/multimer detection systems in the present assessment. In run 43, 53% (117 of 220) of the laboratories used 3-step polymer/multimer systems, that increased to 82% (238 of 291) in the present assessment.

Controls

Fallopian tube and kidney are both recommendable as positive tissue controls when mAb clone WT49 and rmAb clones EP122 and D8I7F are used for WT1 detection. In fallopian tube, the protocol must be calibrated to provide a strong, distinct nuclear staining in virtually all epithelial and smooth muscle cells. In kidney, working as both positive and negative tissue control, optimally calibrated protocols must show a strong, distinct nuclear staining in podocytes and parietal epithelial cells of Bowman's capsule. Epithelial cells of the tubules should not display any nuclear or cytoplasmic staining. When mAb clone 6F-H2 is used with HIER as sole retrieval method, the resulting cytoplasmic staining reaction in endothelial and muscle cells makes kidney less useful as positive tissue (Fig. 7a). On the other hand, if mAb clone 6F-H2 is used with combined HIER-Protease retrieval, the cytoplasmic staining reaction is virtually eliminated and consequently kidney can be very useful as positive tissue control (Fig. 7b).

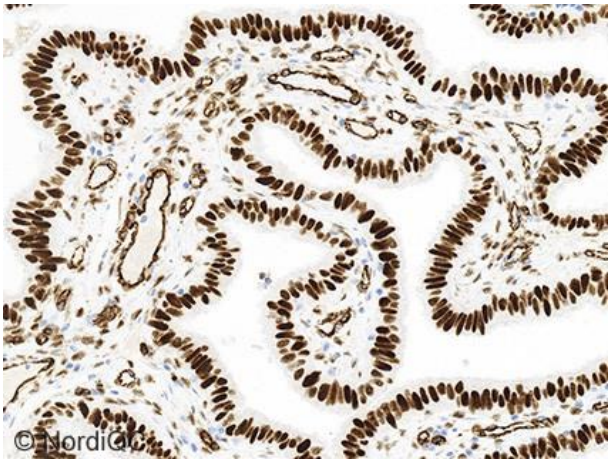


Fig. 1a (x200)
Optimal WT1 staining of fallopian tube, tissue core no. 2, using the **mAb clone 6F-H2** in a RTU format (Dako, IR055/IS055) on the Dako Autostainer platform using HIER in an alkaline buffer and a 3-step polymer detection system (EnVision Flex+). A strong, distinct nuclear staining of virtually all epithelial cells and smooth muscle cells is seen. Moderate cytoplasmic staining of endothelial and smooth muscle cells is also seen and accepted with this clone, making interpretation challenging. Also compare with Figs. 2a-4a, same protocol.

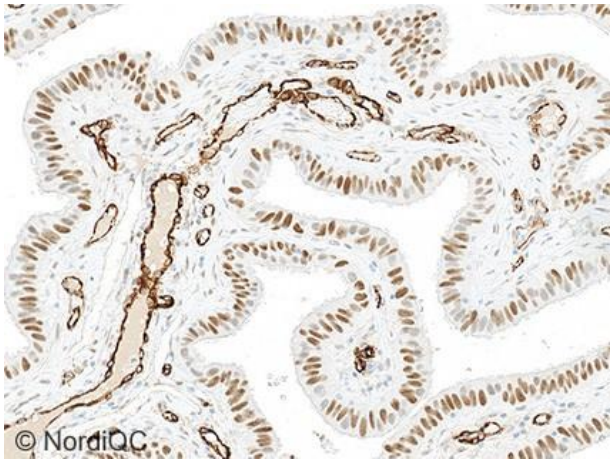


Fig. 1b (x200)
Insufficient WT1 staining of fallopian tube, tissue core no. 2, using the **mAb clone 6F-H2** in a RTU format (Ventana, 760-4397) on the Ventana BenchMark platform using HIER in an alkaline buffer and a 2-step multimer detection system (UltraView). Too short HIER time combined with a relative low sensitivity detection system results in to weak staining results. Weak to moderate nuclear staining in the majority of epithelial cells, whereas only faint nuclear staining is seen in scattered smooth muscle cells. Moderate cytoplasmic staining of endothelial cells is also seen, making interpretation challenging - same field as in Fig. 1a. Also compare with Figs. 2b- 4b, same protocol.

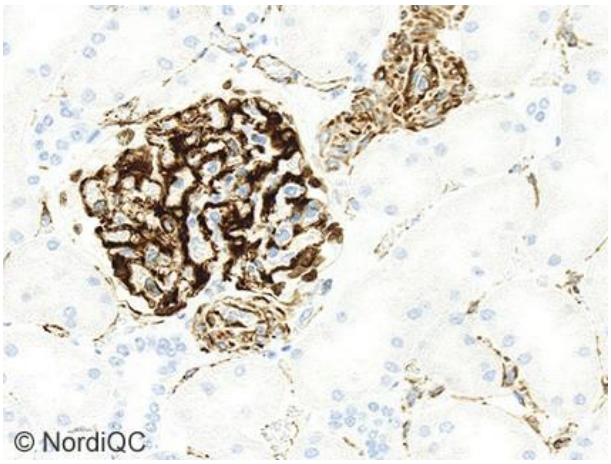


Fig. 2a (x300)
Optimal WT1 staining of kidney, tissue core no. 3, using same protocol as in Fig. 1a. A strong nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen. Moderate cytoplasmic staining of endothelial and muscle cells is also seen, making interpretation very difficult. Also compare with Figs. 3a-4a, same protocol.

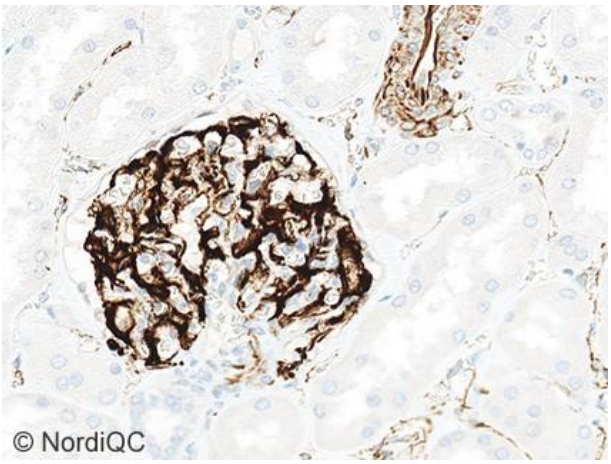


Fig. 2b (x300)
WT1 staining of kidney, tissue core no. 3, using same insufficient staining protocol as in Fig. 1b. A strong nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen. Moderate cytoplasmic staining of endothelial and muscle cells is also seen, making interpretation very difficult – same field as in Fig. 2a. Also compare with Figs. 3b – 4b, same protocol.

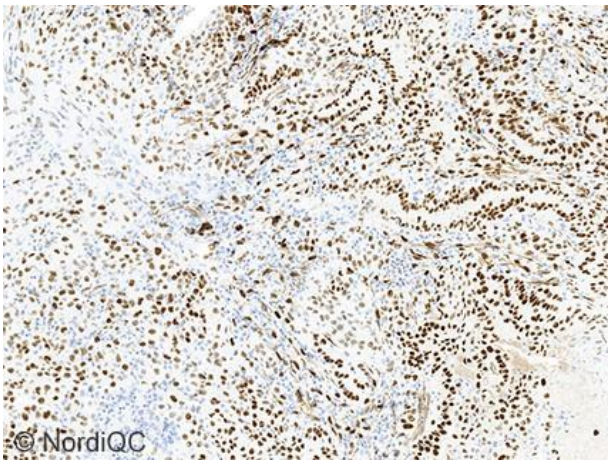


Fig. 3a (x100)
Optimal WT1 staining of mesothelioma, tissue core no. 1, using same protocol as in Fig. 1a and 2a. A strong and distinct nuclear staining of virtually all neoplastic cells is seen. Moderate cytoplasmic staining of endothelial and smooth muscle cells is also seen. Also compare with Fig. 4a, same protocol.

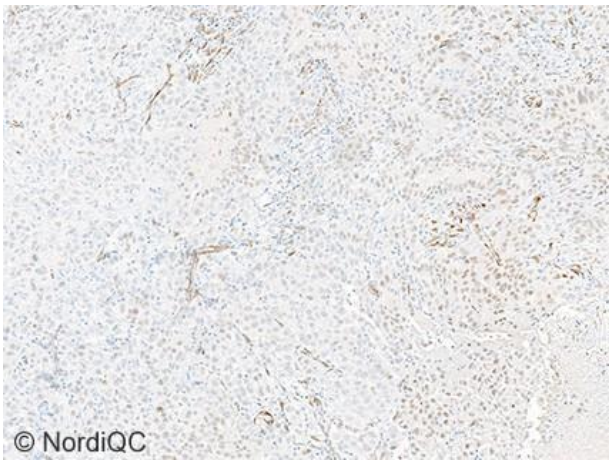


Fig. 3b (x100)
Insufficient WT1 staining of mesothelioma, tissue core no. 1, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 3a. Weak cytoplasmic staining of endothelial and smooth muscle cells is also seen.

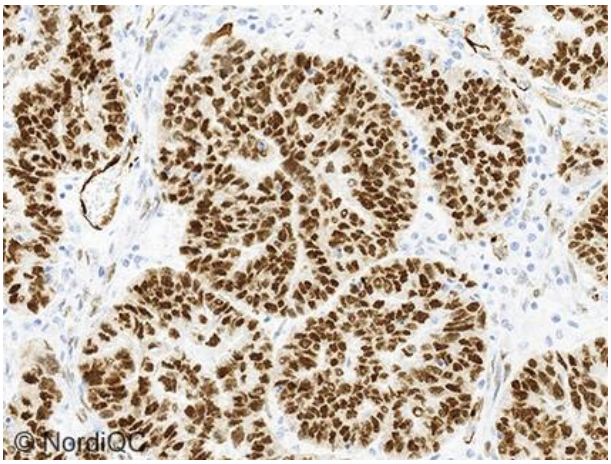


Fig. 4a (x200)
Optimal WT1 staining of the serous ovarian carcinoma, tissue core no. 5, using same protocol as in Fig. 1a-3a. A strong and distinct nuclear staining of virtually all neoplastic cells is seen. Moderate cytoplasmic staining of endothelial and muscle cells is also seen.

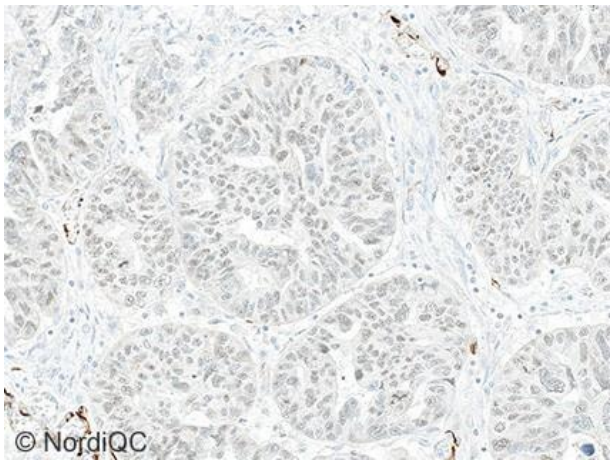


Fig. 4b (x200)
Insufficient WT1 staining of the serous ovarian carcinoma, tissue core no. 5, using same protocol as in Figs. 1b-3b – same field as in Fig. 4a. The proportion and intensity of the neoplastic cells demonstrated is significantly reduced compared to the level expected and obtained in Fig. 4a.

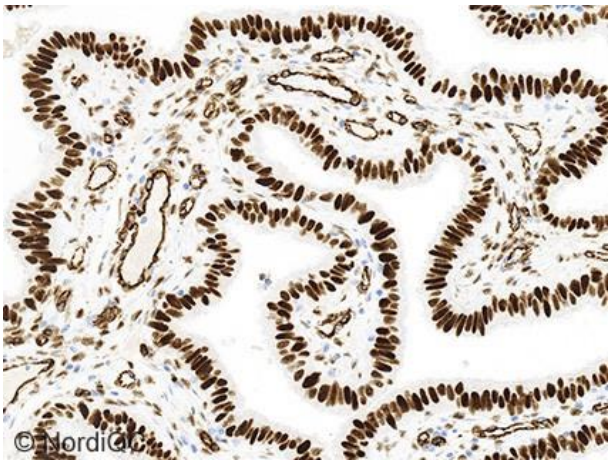


Fig. 5a (x200) same as Fig. 1a
Optimal WT1 staining of fallopian tube, tissue core no. 2, using the **mAb clone 6F-H2** after HIER in alkaline buffer. A strong, distinct nuclear staining of virtually all epithelial cells and smooth muscle cells is seen. Moderate cytoplasmic staining of endothelial and smooth muscle cells is also seen and accepted with this clone, making interpretation challenging. Compare to Figs. 5b, 6a and 6b – same field.

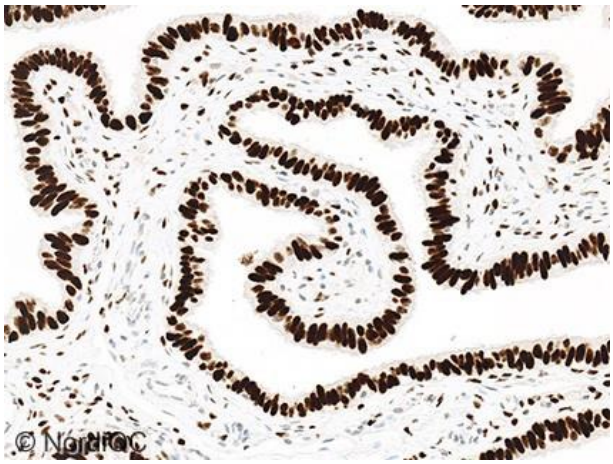


Fig. 5b (x200)
Optimal WT1 staining of fallopian tube, tissue core no. 2, using the **mAb clone 6F-H2** after HIER in alkaline buffer followed by weak proteolysis in Protease 3 (Ventana). A strong, distinct nuclear staining of virtually all epithelial cells and smooth muscle cells is seen and no cytoplasmic staining of endothelial and smooth muscle cells is seen after this combined retrieval, making interpretation much easier. Compare to Figs. 5a, 6a and 6b – same field.

Fig. 6a (x200)
Optimal WT1 staining of fallopian tube, tissue core no. 2, using the **rmAb clone D8I7F** after HIER in alkaline buffer. A strong, distinct nuclear staining of virtually all epithelial cells and smooth muscle cells is seen and no cytoplasmic staining of endothelial and smooth muscle cells is seen with this clone. Compare with Figs. 5a, 5b and 6b – same field.

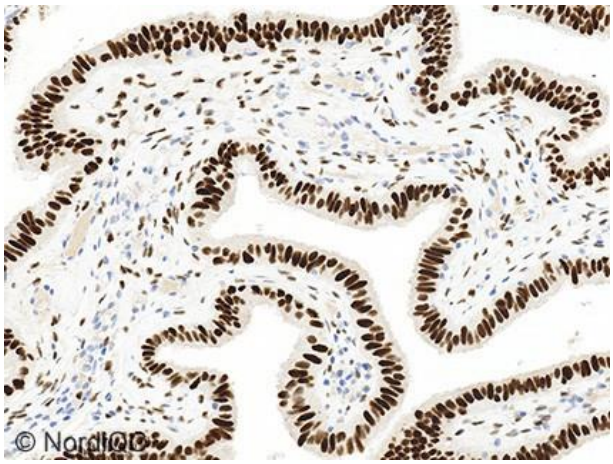


Fig. 6b (x200)
Optimal WT1 staining of fallopian tube, tissue core no. 2, using the **rmAb clone WT49** after HIER in alkaline buffer. A strong, distinct nuclear staining of virtually all epithelial cells and smooth muscle cells is seen and no cytoplasmic staining of endothelial and smooth muscle cells is seen with this clone. Compare with Figs. 5a, 5b and 6a – same field.

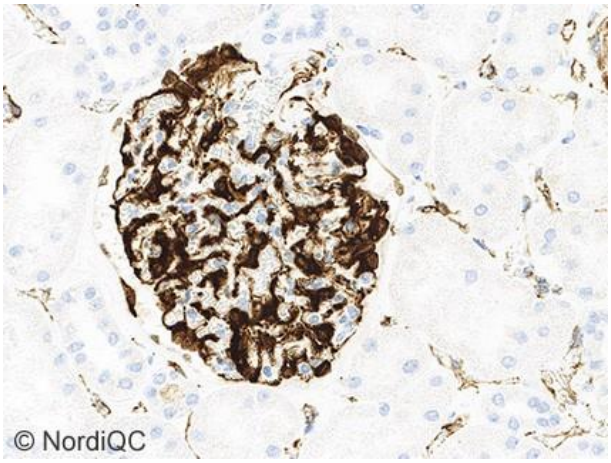


Fig. 7a (x300)

Optimal WT1 staining of kidney, tissue core no. 3, using the mAb clone 6F-H2 after HIER in alkaline buffer. A strong nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen. Moderate cytoplasmic staining of endothelial and smooth muscle cells is also seen, making interpretation very difficult. Compare with Figs. 7b, 8a and 8b – same field.

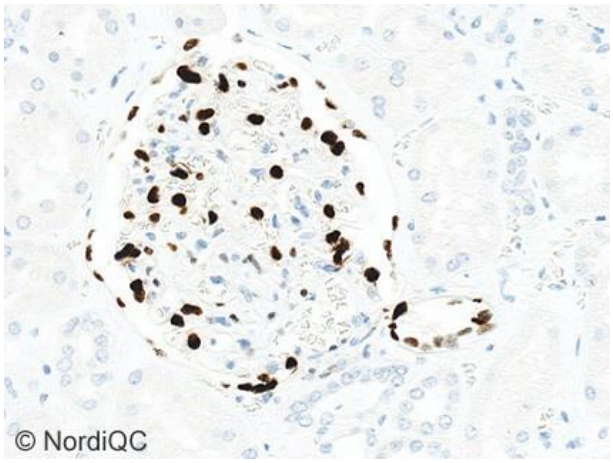


Fig. 7b (x300)
Optimal WT1 staining of kidney, tissue core no. 3, using the mAb clone 6F-H2 after HIER in alkaline buffer followed by weak proteolysis in Protease 3 (Ventana). A strong nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen and no cytoplasmic staining of endothelial and smooth muscle cells is seen after this combined retrieval, making interpretation easier. Compare with Figs. 7a, 8a and 8b – same field.

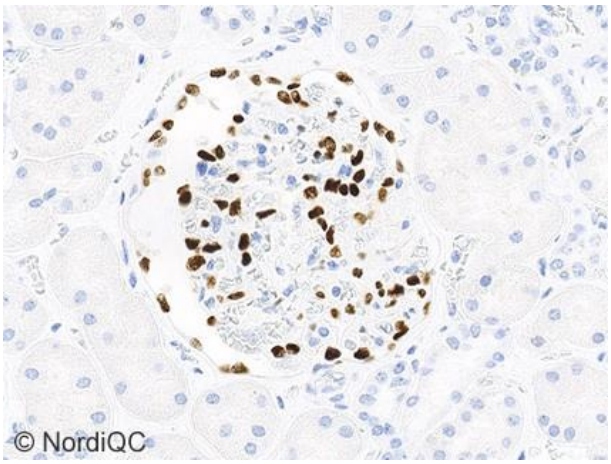


Fig. 8a (x300)
Optimal WT1 staining of kidney, tissue core no. 3, using the **rmAb clone D8I7F** after HIER in alkaline buffer. A strong nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen and no cytoplasmic staining of endothelial and smooth muscle cells is seen with this clone. Compare with Figs. 7a, 7b and 8b – same field.

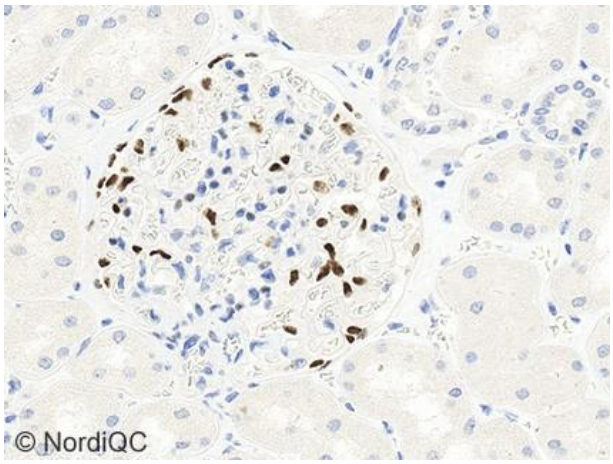


Fig. 8b (x300)
Optimal WT1 staining of kidney, tissue core no. 3, using the **mAb clone WT49** after HIER in alkaline buffer. A strong nuclear staining of the podocytes and the epithelial cells lining the Bowman capsule is seen and no cytoplasmic staining of endothelial and smooth muscle cells is seen with this clone. Compare with Figs. 7a, 7b and 8a – same field.

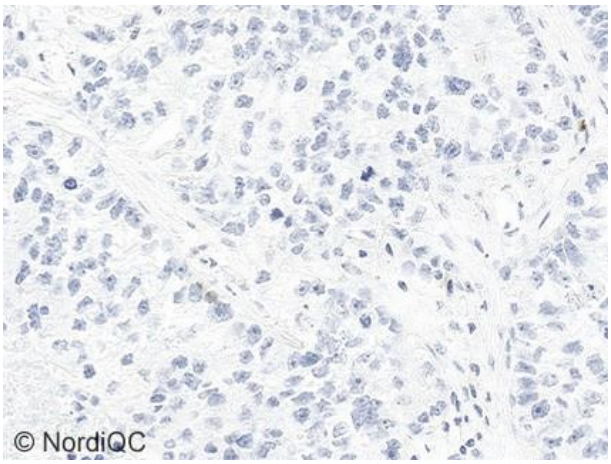


Fig. 9a (x300)
Optimal WT1 staining of lung adenocarcinoma, tissue core no. 4, using the **rmAb clone EP122** after HIER in alkaline buffer. Staining was performed at the Dako Omnis stainer. No staining reaction is seen. Compare with Figs. 9b – same field.

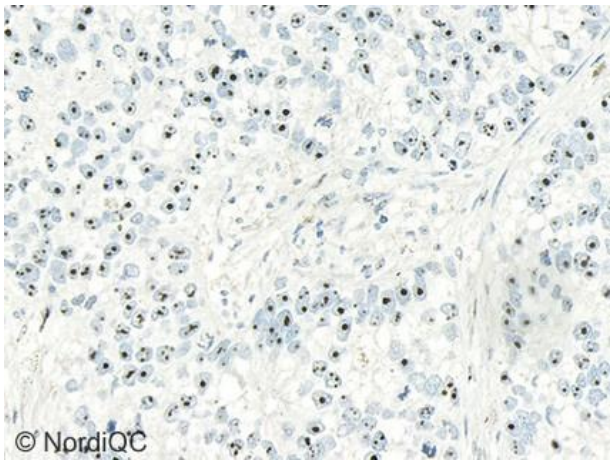


Fig. 9b (x300)
Optimal WT1 staining of lung adenocarcinoma, tissue core no. 4, using the **rmAb clone EP122** after HIER in alkaline buffer. Staining was performed at the Sakura Genie stainer. The majority of cells display a moderate to strong, distinct staining of the nucleoli. The nature of this aberrant staining reaction is unknown, but as it did not interfere with the interpretation of the specific nuclear WT1 reaction, no downgrading was performed on this basis. Compare with Figs. 9a – same field.

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