Assessment Run 63 2021
p53

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of p53 IHC tests among NordiQC participants for the demonstration of corresponding TP53 mutations in endometrial carcinomas. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for p53 (see below).

Material
The slide to be stained for p53 comprised:

1. Endometrial serous carcinoma with p53 overexpression, 2. Endometrial serous carcinoma with absence of p53 expression, 3. Tonsil, 4. Low grade endometrial carcinoma – p53 wild-type, 5. Appendix.

All tissues were fixed in 10% neutral buffered formalin.

Criteria* for assessing a p53 staining as optimal included:

- A weak to moderate nuclear staining reaction in ≥20% of the germinal centre B-cells of the tonsil.
- A weak to moderate nuclear staining reaction in dispersed epithelial cells in the basal crypts of the appendix.
- A moderate to strong, distinct nuclear staining reaction in virtually all the neoplastic cells of the ovarian serous carcinoma with p53 overexpression (tissue core no. 1).
- No staining reaction in the neoplastic cells in the endometrial serous carcinoma with absence of p53 expression (tissue core no. 2). Dispersed stromal cells, lymphocytes and endothelial cells must show an at least weak nuclear staining reaction.
- A weak to moderate, distinct nuclear staining reaction in the majority of neoplastic cells in the low grade endometrial carcinoma (tissue core no. 4). Dispersed stromal cells, lymphocytes and endothelial cells should show an at least weak nuclear staining reaction.
- Nuclear staining of less than 10% of the mantle zone B-cells of the secondary follicles of the tonsil and no staining of the luminal epithelial cells in the appendix.

* The criteria and expected staining patterns were based on the previous NordiQC assessments and the publication by Köbel et al; *Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility. Int J Gynecol Pathol Vol. 38, No. 1 Supplement 1, January 2019, S123-S131*

Participation

Number of laboratories registered for p53, run 63	377
Number of laboratories returning slides	351 (93%)

Results
At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

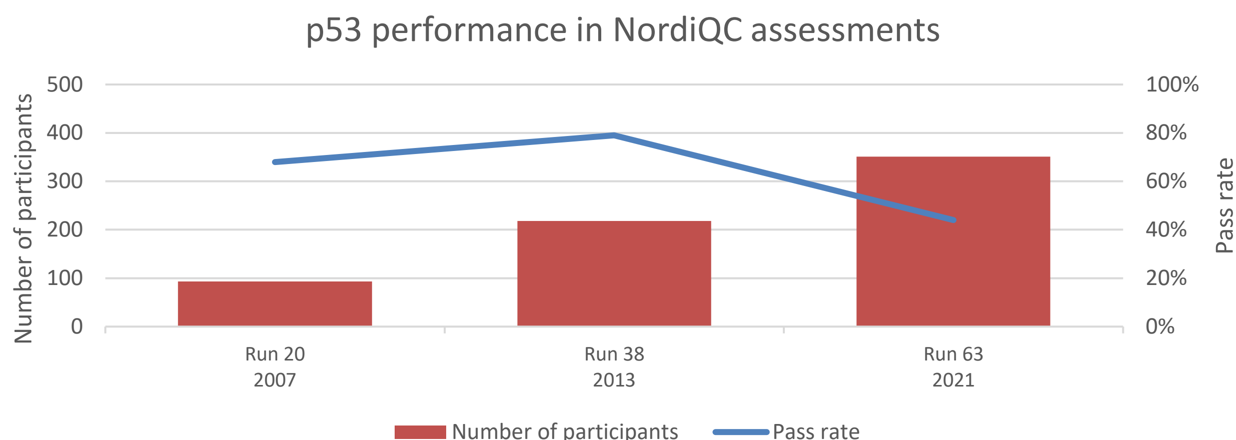
351 laboratories participated in this assessment. 46% achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:

- Use of a less sensitive detection system.
- Too low concentration of the primary antibody.
- Use of a less successful primary Ab.

Performance history
This was the third NordiQC assessment of p53. A significant decrease in the pass rate was seen compared to the previous assessments (see Graph 1). The purpose, scoring criteria in the included neoplasias and composition of the assessment material has changed in this run, and thus might be more challenging than previously.

Graph 1. **Proportion of sufficient results for p53 in the NordiQC runs performed**



Conclusion
The mAb clones **BP53-12** and **DO-7** could both be used to obtain optimal staining result for p53. The most widely used antibody, the mAb clone DO-7 gave optimal staining results on all the main IHC systems from Dako/Agilent, Ventana/Roche and Leica Biosystems. For all the clones efficient HIER, careful calibration of the primary antibody titer and a 3-layer detection system were mandatory for optimal performance. 63% (222 of 351) of the participants used a 3-layer detection system, with an overall pass rate at 68% (150 of 222), 35% optimal (n=78) compared to a pass rate of 9% (12 of 129), 2% optimal (n=3) if using a 2-layer detection system.

Controls
Tonsil and appendix are the most recommendable external positive and negative tissue controls. As a guideline for an accurate p53 IHC test more than 20% of germinal centre B-cells must show a weak to moderate nuclear staining reaction, while less than 10% of the mantle zone B-cells should be demonstrated in tonsil. In appendix, dispersed epithelial cells in the basal parts of the crypts must show a weak to moderate nuclear staining reaction, while the luminal epithelial cells must be negative.
In addition, it has to be emphasized, that stromal cells, lymphocytes and endothelial cells in the clinical samples are essential as internal positive tissue controls especially for carcinomas with TP53 mutations causing absence and loss of p53 expression in the tumour cells.

Table 1. **Antibodies and assessment marks for p53, run 63**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BP53-12**	1 1	BioGenex Zytomed Systems	0	0	1	1	-	-
mAb clone **DO-7**	1 1 7 64 1 23 1 2	Bio SB Immunologic Cell Marque Dako/Agilent Diagnostic BioSystem Leica Biosystems MONOSAN Thermo Scientific	33	23	19	25	56%	33%
mAb clone **DO-7+BP53-12**	2	Thermo Scientific	0	2	0	0	-	-
mAb clone **IHC053**	1	GenomeMe	0	0	1	0	-	-
rmAb clone **ZR153**	1	Zeta Corporation	0	0	0	1	-	-
rmAb clone **QR025**	1	Quartett	0	0	0	1	-	-
Ab clone **BPM6168**	1	Biolynx Biotechnology	0	1	0	0	-	-
Ready-To-Use Antibodies								
mAb clone **BP53-11 760-2542 (VRPS)**[3]	3	Ventana/Roche	0	0	1	2	-	-
mAb clone **BP53-11 760-2542 (LMPS)**[4]	36	Ventana/Roche	7	10	10	9	47%	19%
mAb clone **DO-7 PM042**	1	Biocare Medical	0	0	0	1	-	-

mAb clone **DO-7** **453M-9x**	1	Cell Marque	0	0	0	1	-	-
mAb clone **DO-7** **GA616 (VRPS)[3]**	28	Dako/Agilent	0	0	2	26	0%	0%
mAb clone **DO-7** **GA616 (LMPS)[4]**	34	Dako/Agilent	15	10	2	7	74%	44%
mAb clone **DO-7** **IS/IR616 (VRPS)[3]**	10	Dako/Agilent	1	0	1	8	10%	10%
mAb clone **DO-7** **IS/IR616 (LMPS)[4]**	16	Dako/Agilent	2	5	2	7	44%	13%
mAb clone **DO-7** **A00021**	1	ScyTek Laboratories	0	0	1	0	-	-
mAb clone **DO-7** **PA0057 (VRPS)[3]**	14	Leica Biosystems	1	6	5	2	50%	7%
mAb clone **DO-7** **PA0057 (LMPS)[4]**	7	Leica Biosystems	2	4	1	0	86%	29%
mAb clone **DO-7** **800-2912 (VRPS)[3]**	5	Ventana/Roche	1	2	0	2	60%	20%
mAb clone **DO-7** **800-2912 (LMPS)[4]**	81	Ventana/Roche	18	18	24	21	44%	22%
mAb clone **BP53-12** **BMS064**	1	Zytomed Systems	0	0	0	1	-	-
mAb clone **C2H10** **CPM-0141**	1	Celnovte	1	0	0	0	-	-
rmAb clone **EP9** **AN728-5M**	1	BioGenex	0	0	0	1	-	-
rmAb clone **SP5** **MAD-000309QD**	3	Master Diagnostica	0	0	0	3	-	-
Total	351		81	81	70	119		
Proportion			23%	23%	20%	34%	46%	

1) Proportion of sufficient stains (optimal or good) (≥5 asessed protocols).
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **DO-7**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1; Ventana/Roche) (20/52)*, Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) (1/9), Bond Epitope Retrieval 2 (BERS2; Leica Biosystems) (6/15), Target Retrieval Solution (TRS) pH 9 (Dako/Agilent) (5/11) or TRS pH 6.1 (3-in-1) (Dako/Agilent) (1/2) as retrieval buffer. The mAb was diluted in the range of 1:80 -1:1000 depending on the total sensitivity of the protocol employed. Using these protocol settings 51 of 83 (61%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

Table 2. **Optimal results for p53 using concentrated antibodies on the main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer Link / Classic**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark GX / XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **DO-7**	0/5** (0%)	1/2	5/10 (50%)	-	20/42 (48%)	-	6/13 (46%)	1/7 (14%)

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

Ready-To-Use Antibodies
mAb clone **Bp53-11,** product no. **760-2542**, Ventana/Roche, BenchMark XT/Ultra:
Protocols with optimal results were based on HIER using CC1, efficient heating time 24-64 min. and 12-32 min. incubation of the primary Ab. OptiView (760-700) or UltraView (760-500) with amplification (760-080) were used as detection system. Using these protocol settings 13 of 17 (76%) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7**, product no. **IS/IR616**, Dako/Agilent, Dako Autostainer:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1), efficient heating time 20 min. at 95-97°C, 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 5 of 15 (67%) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7**, product no. **GA616**, Dako/Agilent, Dako Omnis:
Protocols with optimal results were typically based on HIER in PT-Link using TRS High pH, efficient heating time 24-30 min., and 15-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823) as detection system. Using these protocol settings 24 of 25 (96%) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7** product no. **PA0057**, Leica Biosystems, Leica Bond III:
Protocols with optimal results were based on HIER using BERS2, efficient heating time 20 min., 15-30 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 10 of 17 (59%) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7** product no. **800-2912**, Ventana/Roche, BenchMark XT/Ultra/GX:
Protocols with optimal results were typically based on HIER using CC1, efficient heating time 32-64 min., 12-40 min. incubation of the primary Ab and OptiView (760-700) or UltraView (760-500) with amplification (760-080) as detection system. Using these protocol settings 33 of 48 (69%) laboratories produced a sufficient staining (optimal or good).

Table 3 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 3. **Proportion of sufficient and optimal results for p53 for the most commonly used RTU IHC systems**

RTU systems	**Vendor recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Ventana Benchmark mAb clone **Bp53-11, 760-2542**	(0/3)	(0/3)	47% (17/36)	19% (7/36)
Dako Autostainer mAb clone **DO-7, IR/IS616**	10% (1/10)	10% (1/10)	42% (5/12)	17% (2/12)
Dako Omnis mAb clone **DO-7, GA616**	0% (0/28)	0% (0/28)	78% (25/32)	47% (15/32)
Leica Bond mAb clone **DO-7, PA0057**	50% (7/14)	7% (1/14)	100% (5/5)	20% (1/5)
Ventana Benchmark mAb clone **DO-7, 800-2912**	60% (3/5)	20% (1/5)	44% (36/81)	22% (18/81)

* 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 this assessment and in concordance with the previous assessment of p53, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of the cells and structures expected to be demonstrated. Too weak or a complete false negative staining reaction was seen in 96% of the insufficient results (181 of 189). The remaining 4% (8 of 189) insufficient results were characterized by either a false positive staining reaction (n=4) or poor signal-to-noise ratio/excessive background (n=4). Virtually all laboratories were able to demonstrate p53 in the endometrial serous carcinoma with p53 overexpression (tissue core no. 1) and high-level antigen level. On the contrary, the demonstration of p53 in low-level structures as stromal cells, lymphocytes and endothelial cells in the endometrial serous carcinoma with loss of p53 (tissue core no. 2), the neoplastic cells in the p53 wild-type low grade

endometrial carcinoma (tissue core no. 4) and germinal centre and mantle zone B-cells in tonsil were much more challenging and required a carefully calibrated protocol.

31% (108 of 351) of the laboratories used an Ab as concentrated format within a laboratory developed (LD) assay for p53. 93% (100 of 108) of the LD assays were based on the mAb clone DO-7 with a relatively low pass rate at 56%. However, optimal results could be obtained on all four main IHC systems (see Table 2). The main prerequisites for sufficient staining results were HIER in an alkaline buffer, careful calibration of the titre of the primary Ab and use of sensitive detection system. If using a 3-layer polymer/multimer based system as EnVision FLEX+ (Dako/Agilent), OptiView (Ventana/Roche) or Refine (Leica Biosystems), a pass rate of 69% (49 of 71) was observed, 44% optimal (n=31) compared to a pass rate of 24% (7 of 29), 7% optimal (n=2), if using a 2-step detection system as EnVision FLEX (Dako/Agilent) or UltraView (Ventana/Roche).

Ready-To-Use (RTU) antibodies were used by 69% (243 of 351) of the laboratories.
Overall, it was observed that the pass rates and proportion of optimal results were low for the RTU systems from the three main IHC providers, Dako/Agilent, Ventana/Roche and Leica Biosystems, when these were applied by vendor recommended protocol settings (VRPS) - see Tables 1 and 3. If the RTU systems from these three vendors were used by VRPS an overall pass rate of 18% was seen and only 5% optimal. This observation and less successful performance were in clear contrast to the result obtained in the last NordiQC assessment for p53 in which the same RTU systems were very successful with pass rates in between 85-100%. The discrepancy is most likely influenced by the altered focus for usage of IHC for p53 in endometrial carcinomas and awareness of more TP53 mutations being present with different p53 expression patterns. Previously the intended use of IHC for p53 mainly focused on the demonstration of p53 overexpression caused by TP53 mutations, but at present also the TP53 mutations with loss of p53 expression must be identified. In the latter an increased demand for the p53 IHC test also to consistently demonstrate p53 expression in internal cells is induced and a recalibration of the IHC test typically must be performed.

The Leica Biosystems RTU systems based on mAb clone DO-7 was the most successful RTU system if modifying the protocol settings with a pass rate at 100% compared to 50% if following the vendor recommended protocol settings (see Table 3). The vendor recommended protocol was based on HIER in BERS2 for 20 min., 15 min. incubation of the primary Ab and Refine as detection system. Minor adjustments in incubation times for HIER and primary Ab was seen for the modified protocols.

The Dako/Agilent RTU system for Omnis, based on mAb clone DO-7 was most successful if modifying the protocol settings, and thus obtained a pass rate at 78% (25 of 32). However, if following the vendor recommended protocol settings, no sufficient staining results were seen (0 of 28) (see Table 3). The vendor recommended protocol was based on HIER in TRS High pH for 30 min., 20 min. incubation of the primary Ab and EnVision Flex as detection system. The most successful modification was adding a mouse linker to the detection system. If using EnVision Flex+, a pass rate at 92% (24 of 26) was obtained.
The same pattern was seen for the Dako Autostainer RTU system, also based on mAb clone DO-7. If using the 3-layer EnVision Flex+, a pass rate 100% (5 of 5) was observed, compared to 6% (1 of 17) if using EnVision Flex as detection system.

The Ventana/Roche RTU system based on mAb clone DO-7 was the most widely used RTU system. The VRPS were based on either UltraView or OptiView as detection system. Using UltraView, the protocol was based on HIER in CC1 for 60 min. and primary Ab incubation time of 24 or 28 min. for BenchMark Ultra or XT, respectively. Using OptiView, the protocol was based on HIER in CC1 for 32 min. and primary Ab incubation time of 16 min. The majority of laboratories modified the protocol settings. The most common modifications were prolonging HIER and incubation time of primary Ab. If using UltraView, no sufficient staining results were seen. If using OptiView or UltraView with Amplification, a pass rate at 61% (39 of 64) was obtained.

The Ventana/Roche RTU system based on the mAb clone Bp53-11 obtained a pass rate at 44% (17 of 39). Three laboratories unsuccessfully used the vendor recommended protocol settings. The VRPS were based on either UltraView or OptiView as detection system. Using UltraView, the protocol was based on HIER in CC1 for 36 min. and primary Ab incubation time of 24 or 32 min. for BenchMark Ultra or XT, respectively. Using OptiView, the protocol was based on HIER in CC1 for 32 min. and primary Ab incubation time of 12 or 16 min. for BenchMark Ultra or XT, respectively. The most common and successful modification was related to a prolonged incubation time of primary Ab. In general, the use of UltraView as detection system was found less successful. A pass rate at 11% (2 of 18) was seen when using UltraView compared to 71% (15 of 21) if using OptiView or UltraView with Amplification.

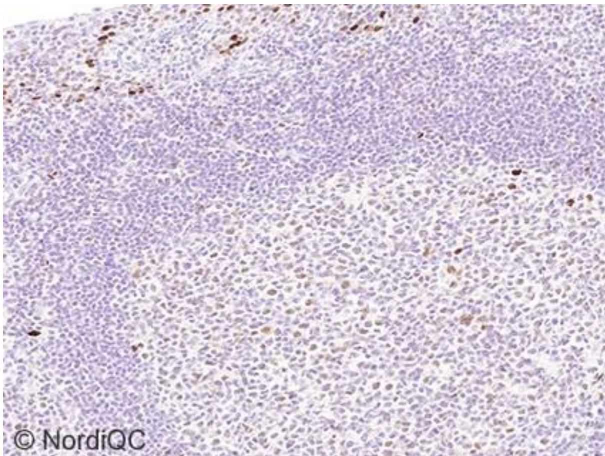


Fig. 1a
Optimal p53 staining of the tonsil using the mAb clone DO-7 as RTU for Dako Omnis, Dako/Agilent, using modified protocol settings with HIER in an alkaline buffer and EnVision Flex+ as detection system.
A weak to moderate nuclear staining reaction is seen in >20 % of the germinal centre B-cells, whereas <10 % of the mantle zone B-cells are demonstrated. Scattered squamous epithelial cells show a moderate to strong nuclear staining reaction. Also compare with Figs. 2a-5a, same protocol.

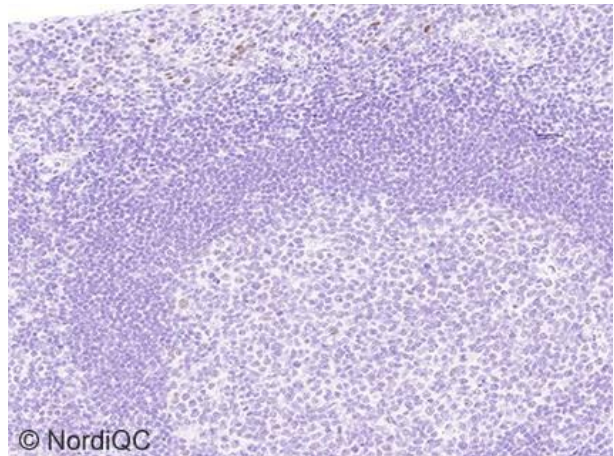


Fig. 1b
Insufficient p53 staining of the tonsil using the mAb clone DO-7 as RTU for Dako Omnis, Dako/Agilent, using the recommended protocol settings with HIER in an alkaline buffer and EnVision Flex as detection system.
Virtually no nuclear staining reaction for p53 is seen in neither the germinal centre B-cells or in the squamous epithelial cells compared to the optimal result in Fig. 1a – same area. Also compare with Figs. 2b-5b, same protocol.

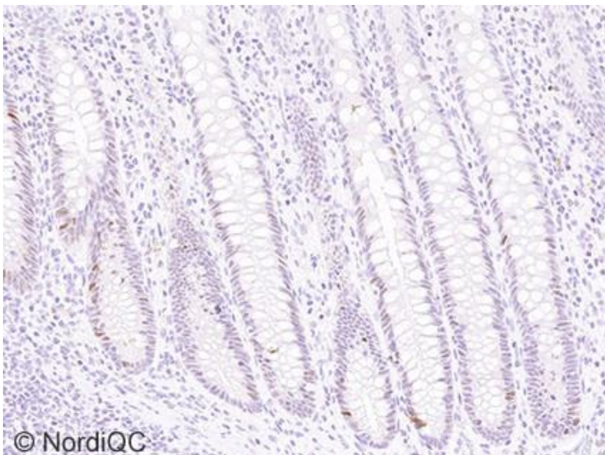


Fig. 2a
Optimal p53 staining of the appendix using same protocol as in Fig. 1a.
Dispersed epithelial cells of the basal parts of the crypts show a weak to moderate nuclear staining reaction.

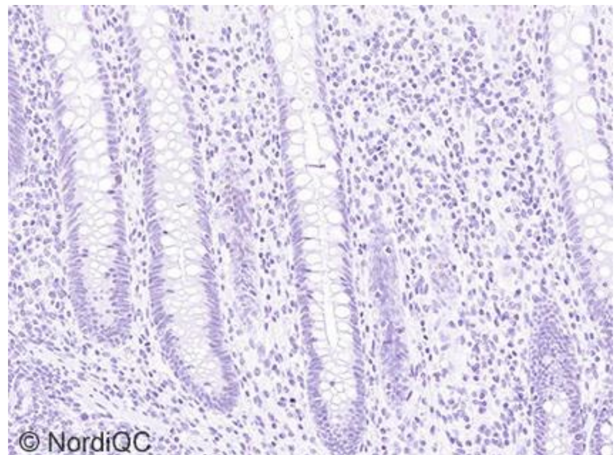


Fig. 2b
Insufficient p53 staining of the appendix using same protocol as in Fig. 1b.
Virtually no nuclear staining reaction for p53 is seen in the crypt epithelial cells compared to the optimal result in Fig. 2a – same area. Also compare with Figs. 3b-5b, same protocol.

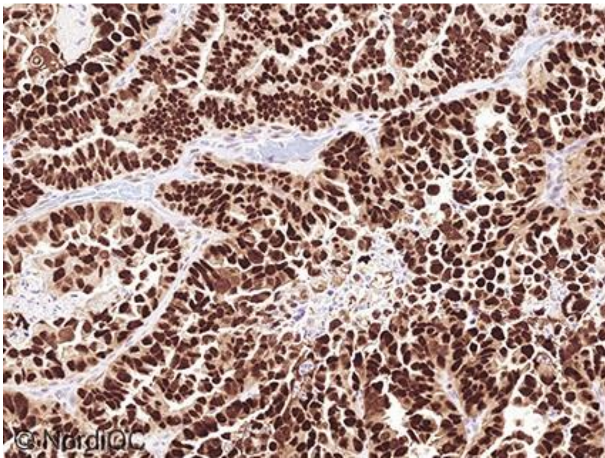


Fig. 3a
Optimal p53 staining of the endometrial serous carcinoma with p53 overexpression (tissue core no. 1), using same protocol as in Figs. 1a – 2a.
Virtually all neoplastic cells show a strong, nuclear staining reaction.

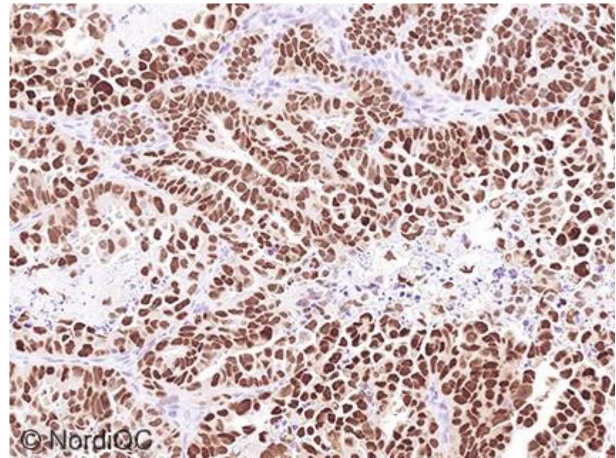


Fig. 3b
p53 staining of the endometrial serous carcinoma with p53 overexpression (tissue core no. 1), using same protocol as in Figs. 1b – 2b.
Virtually all neoplastic cells show a moderate to strong, nuclear staining reaction. The intensity is slightly reduced compared to the optimal result in Fig. 3a – same area, but overall, all cells are demonstrated as these have high-level p53 expression.

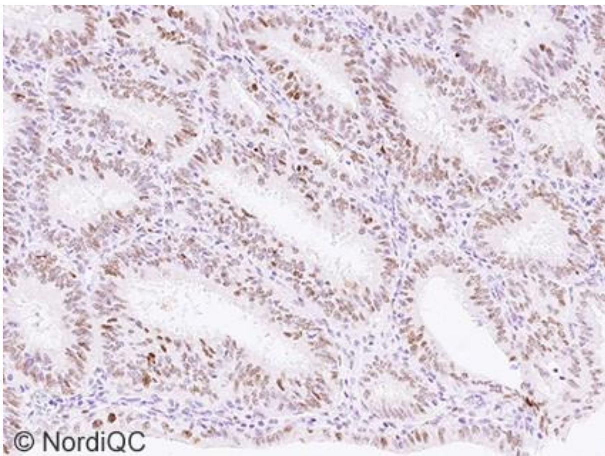


Fig. 4a
Optimal p53 staining of the low grade endometrial carcinoma, p53 wild-type (tissue core no. 4), using same protocol as in Figs. 1a – 3a.
Virtually all neoplastic cells show a weak to moderate, nuclear staining reaction.

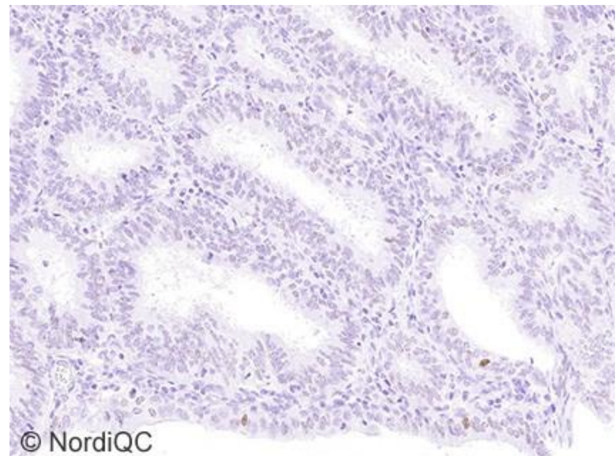


Fig. 4b
Insufficient p53 staining of the low grade endometrial carcinoma, p53 wild-type (tissue core no. 4), using same protocol as in Figs. 1b – 3b.
Virtually no nuclear staining reaction for p53 is seen in the neoplastic cells compared to the optimal result in Fig. 4a – same area.

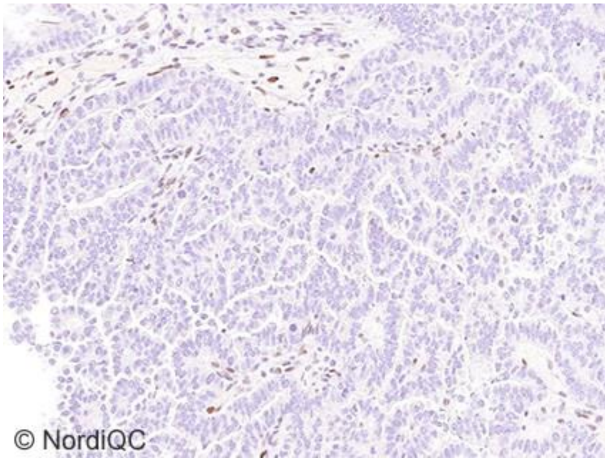


Fig. 5a
Optimal p53 staining of the endometrial serous carcinoma with absence of p53 expression (tissue core no. 2), using same protocol as in Figs. 1a – 4a.
No nuclear staining reaction is seen in the neoplastic cells. Stromal cells are weakly positive, serving as internal positive tissue control.

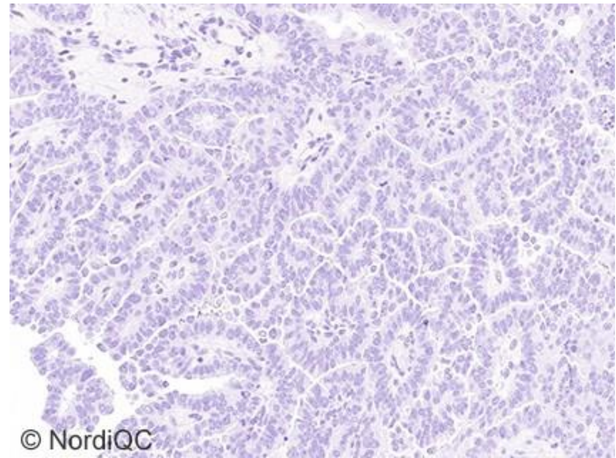


Fig. 5b
Insufficient p53 staining of the endometrial serous carcinoma with absence of p53 expression (tissue core no. 2), using same protocol as in Figs. 1b – 4b.
Virtually no nuclear staining reaction for p53 is seen in neither the neoplastic or stromal cells.

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