Assessment Run 38 2013
p53

Material
The slide to be stained for p53 comprised:

1. Colon, 2. Fallopian tube, 3. Tonsil, 4. Colon adenocarcinoma,
5. Ovarian serous carcinoma, 6. Urothelial carcinoma

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 scattered epithelial cells of the Fallopian tube and 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 and the colon adenocarcinoma
- A moderate to strong, distinct nuclear staining reaction in the majority of the neoplastic cells of the urothelial carcinoma
- 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 colon.

218 laboratories participated in this assessment. 79 % achieved a sufficient mark (optimal or good). Antibodies (Abs) used and marks are summarized in table 1.

Table 1. **Antibodies and assessment marks for p53, run 38**

Concentrated Antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BP53-12**	2 1 1	Biogenex Genemed Zytomed	2	0	0	2	-	-
mAb clone **CC53**	1	Dianova	1	0	0	0	-	-
mAb clone **DO-1**	2	Santa Cruz	0	2	0	0	-	-
mAb clone **DO-7**	82 15 6 2 2 1 1	Dako Leica/Novocastra Thermo/Neomarkers Biocare Cell Marque Monosan Vector	41	43	22	3	77 %	82 %
mAb clone **DO-7+BP53-12**	7	Thermo/Neomarkers	2	4	1	0	86 %	100 %
rmAb clone **318-6-11**	3	Dako	1	1	1	0	-	-
rmAb clone **EP9**	1	Epitomics	0	0	1	0	-	-
rmAb clone **SP5**	1 1	Spring Bioscience Thermo/Neomarkers	0	2	0	0	-	-
pAb **NCL-P53-CM1**	1	Leica/Novocastra	0	0	1	0	-	-
Unknown	1	Unknown	0	1	0	0	-	-
Ready-To-Use Antibodies								
mAb clone **Bp53-11 760-2542**	8	Ventana	2	6	0	0	100 %	100 %
mAb clone **DO-7**	1		0	1	0	0	-	-

PM042		Biocare						
mAb clone **DO-7 453M-9x**	1	Cell Marque	0	0	0	1	-	-
mAb clone **DO-7 IS/IR616**	33	Dako	8	21	4	0	88 %	90 %
mAb clone **DO-7 N1581**	3	Dako	0	2	1	0	-	-
mAb clone **DO-7 GM700102**	1	Gene Tech	0	1	0	0	-	-
mAb clone **DO-7 PA0057**	7	Leica	5	2	0	0	100 %	100 %
mAb clone **DO-7 MON-RTU1168**	1	Monosan	0	0	1	0	-	-
mAb clone **DO-7 790/800-2912**	27	Ventana	13	10	4	0	85 %	91 %
mAb clone **DO-7 ZM-0408**	2	Zhongshan	1	0	1	0	-	-
rmAb clone **SP5 453R-17**	1	Cell Marque	0	0	1	0		
rmAb clone **SP5 MAD-000309QD**	1	Master Diagnostica	0	0	1	0	-	-
rmAb **Y5 PME298**	1	Biocare	0	0	1	0	-	-
Total	218		76	96	40	6	-	
Proportion			35 %	44 %	18 %	3 %	79 %	

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

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **BP53-12**: Two protocols with optimal results were both based on heat induced epitope retrieval (HIER) using Cell Conditioning 1 (CC1; Ventana) (1/2)* or Bond Epitope Retrieval Solution 1 (BERS1; Leica) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:100 -1:15.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 of 3 (67 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **CC53**: The protocol with an optimal result was based on HIER using CC1 (BenchMark, Ventana) as retrieval buffer. The mAb was diluted 1:100.

mAb clone **DO-7**: Protocols with optimal results were all based on HIER using either TRS pH 9 (3-in-1) (Dako) (7/20), TRS pH 9 (Dako) (1/5), CC1 (BenchMark, Ventana) (18/43), CC2 (BenchMark, Ventana) (1/3), BERS 2 (Leica) (2/6), BERS 1 (Leica) (5/8), Tris-EDTA/EGTA pH 9 (5/8), EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (1/4) as retrieval buffer. The mAb was typically diluted in the range of 1:80-1:2.400 depending on the total sensitivity of the protocol employed. Using these protocol settings 69 of 84 (82 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7+BP53-12**: Two protocols with optimal results were both based on HIER using either Tris-EDTA/EGTA pH 9 (1/1) or Citrate pH 6 (1/1) as retrieval buffer. The mAb was diluted in the range of 1:400-1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 of 2 (100 %) produced an optimal staining.

Table 2. O**ptimal results for p53 using concentrated antibodies on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **DO-7**	42 % 10/24*	0 % 0/1	45 % 15/33	33 % 1/3	40 % 2/5	71 % 5/7

* 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** (prod. no. 760-2542, Ventana): Protocols with optimal results were based on HIER using mild or standard Cell Conditioning 1, 28-32 min incubation of the primary Ab and UltraView (760-500) as detection system. Using these protocol settings 3 of 3 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7** (product.no. IS/IR616, Dako): Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) or TRS pH 9 (efficient heating time 10-20 min at 95-99°C) 20-30 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 27 of 30 (90 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7** (prod. no. PA0057, Leica): Protocols with optimal results were all based on HIER using BERS 2 (Bond, Leica), 15 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 6 of 6 (100 %) laboratories produced a sufficient staining (optimal or good).

mAb clone **DO-7** (prod. no. 790/800-2912, Ventana): Protocols with optimal results were all based on HIER using mild or standard Cell Conditioning 1, 8-32 min incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection system. Using these protocol settings 21 of 23 (91 %) laboratories produced a sufficient staining (optimal or good).

The most frequent causes of insufficient stainings were:

- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Less successful primary antibody / Ready-To-use format

In this assessment and in concordance with the previous assessment of p53 (run 20) the prevalent feature of an insufficient staining was a too weak or false negative staining of p53. Too weak or false negative staining was seen in 61 % of the insufficient results (28 of 46)and was characterized by a weak staining intensity and reduced proportion of neoplastic cells demonstrated in the urothelial carcinoma and in the normal tonsilar germinal centre B-cells. Typically, these weak staining results were caused by a too low concentration of the primary Ab, including use of Ready-To-Use (RTU) formats not calibrated properly by the vendors or not being designed to be used within a complete IHC system. In the remaining 39 % false positive staining was seen, typically characterized by a nuclear staining of virtually all cells inclusive the resting mantle zone B-cells. In this assessment a cut-off value of 10 % positivity of the mantle zone B-cells was applied as threshold. The false positive staining results were typically caused by a too high concentration of the primary Ab in combination with efficient HIER and a 3-step polymer/multimer based detection system.

The most widely used mAb clone was DO-7, which applied as a concentrate with HIER resulted in a high proportion of sufficient results. Using this clone, optimal staining results could be obtained on all 3 main IHC platforms from Dako, Ventana and Leica (see table 2).
Corresponding RTU systems, all based on the mAb clone DO-7, showed a slightly superior pass rate compared to the laboratory validated assays based on the same clone. Using the mAb clone DO-7 by laboratory validated assays a pass rate of 82 % was seen, compared to a pass rate of 100 %, 91 % and 90 % using the RTU systems from Leica, Ventana and Dako, respectively.

Controls

In this assessment tonsil and colon were identified as the most recommendable positive and negative tissue controls. In tonsil, morethan 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 colon, 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.

Performance history
This was the 2nd NordiQC assessment of p53. Increase in the pass rate was seen compared to the previous run 20, 2007 (se table 3).

Table 3. **Proportion of sufficient results for p53 in the two NordiQC runs performed**

	Run 20 2007	Run 38 2013
Participants, n=	93	218
Sufficient results	68 %	79 %

It is difficult to conclude on the causes for the improvement as many laboratories participated for the first time and the vast majority of the laboratories participating in the previous assessment have modified their protocols compared to the protocols used in run 20, 2007. However the improved pass rate was seen to be related to the high quality and extended use of the Ready-To-Use (RTU) systems for p53 from the three main providers Ventana, Dako and Leica as the RTU systems from these companies in this assessment showed a superior pass-rate of 90 -100 % compared to the pass-rates for the in-house validated protocols for p53.

Conclusion
The mAb clones **BP53-12**, **CC53**, **DO-7** and the rmAb clone **318-6-11** can all be used to obtain optimal staining result for p53. The most widely used antibody, the mAb clone DO-7 gave an optimal result on all the 3 main IHC systems from Dako, Ventana and Leica respectively. For all the clones HIER and careful calibration of the primary antibody titer is mandatory for optimal performance.
Tonsil is recommended as positive and negative tissue control for p53: more than 20 % of the germinal centre B-cells must show weak to moderate nuclear staining reaction, while less than 10 % of the mantle zone B-cells must be demonstrated.

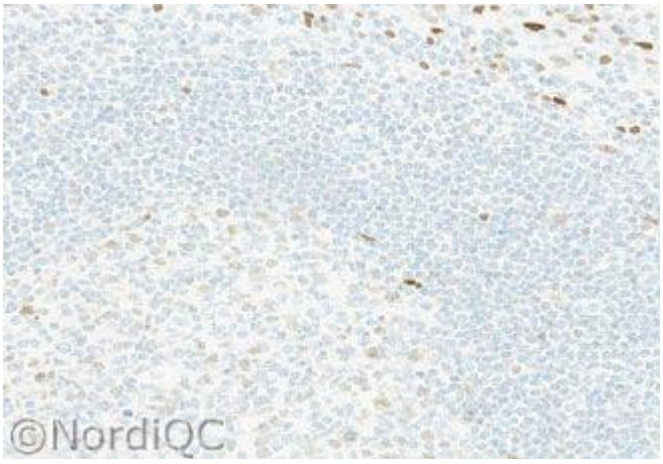

Fig. 1a
Optimal p53 staining of the tonsil using the mAb clone DO-7 as a concentrate optimally calibrated and with HIER in an alkaline buffer and performed on the BenchMark ULTRA, Ventana.
A weak to moderate nuclear staining reaction is seen in > 20 % of the germinal centre B-cells, whereas < 10 % of the mantle zone B-cells are demonstrated. In the right corner, top, scattered squamous epithelial cells show a moderate to strong nuclear staining reaction.
Also compare with Figs. 1b, 2a and 2b – same protocol.

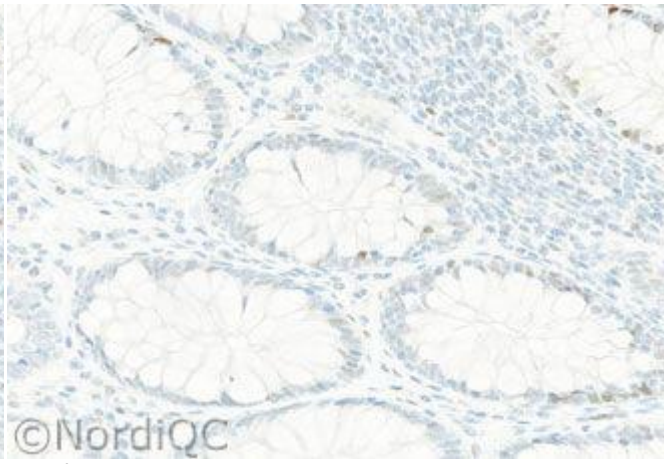

Fig. 1b
Optimal p53 staining of the appendix using same protocol as in Fig. 1a.
Dispersed epithelial cells of the basal parts of the crypts show a weak to moderate nuclear staining reaction.
Virtually all the stromal cells are negative.

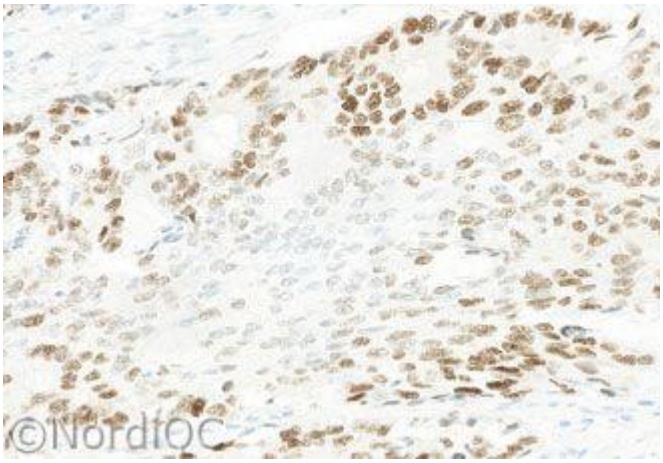

Fig. 2a
Optimal p53 staining of the colon adenocarcinoma using same protocol as in Figs. 1a. and 1b.
A moderate to strong nuclear staining reaction is seen the majority of the neoplastic cells.
No nuclear staining reaction is seen in the stromal cells.

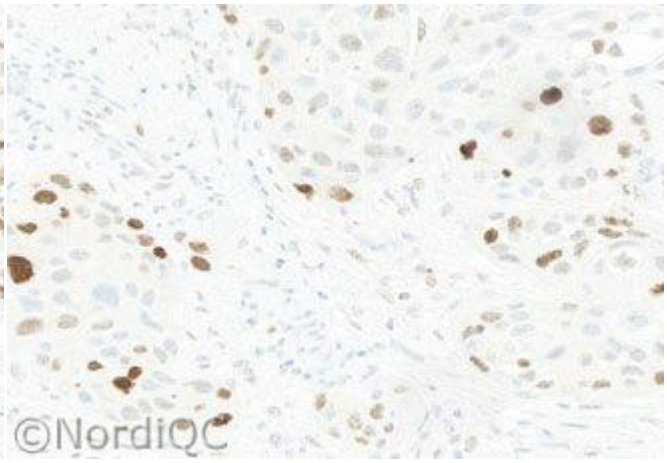

Fig. 2b
Optimal p53 staining of the urothelial carcinoma using same protocol as in Figs. 1a, 1b. and 2a.
A weak to strong nuclear staining reaction is seen the majority of the neoplastic cells.
No nuclear staining reaction is seen in the stromal cells.

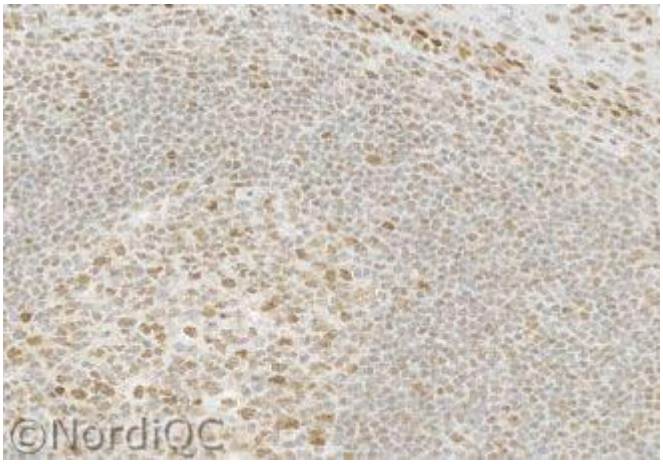

Fig. 3a
Insufficient false positive p53 staining of the tonsil using the mAb clone DO-7 by protocol settings giving a too high sensitivity – most likely due to a too high concentration of the primary Ab combined with HIER in an alkaline buffer and a 3-step polymer based detection kit – same field as in Fig. 1a.
Virtually all cells, both the proliferating germinal centre B-cells and the dormant mantle zone B-cells show a moderate nuclear staining reaction.
Also compare with Fig. 3b – same protocol.

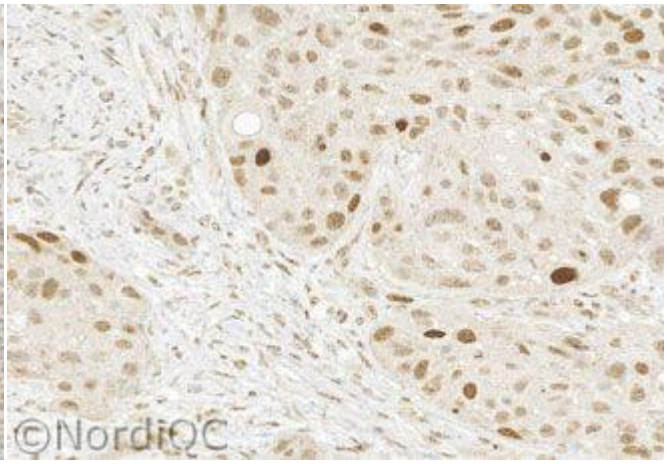

Fig. 3b
Insufficient false positive p53 staining of the urothelial carcinoma using same protocol as in Fig. 3b. – same field as in Fig. 2b.
Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. However at the same time, also all the stromal cells show a distinct nuclear staining reaction.

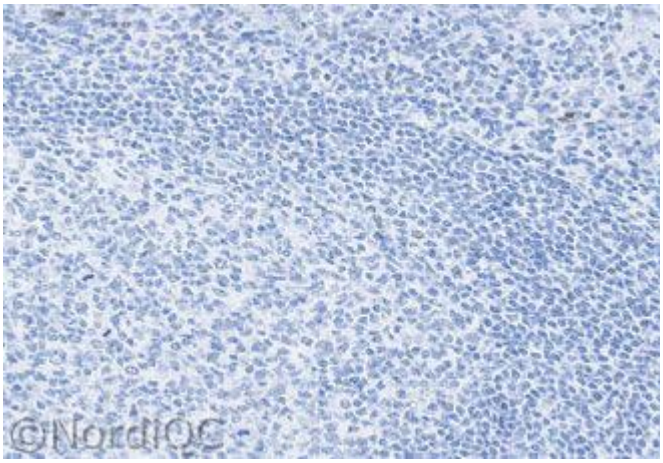

Fig. 4a
Insufficient false negative p53 staining using the mAb clone DO-7 too diluted in combination with an excessive nuclear counterstaining complicating the interpretation. Virtually no nuclear staining reaction for p53 is seen in neither the germinal centre B-cells or in the squamous epithelial cells – same field as in Fig. 1a.
Also compare with Fig. 4b – same protocol.

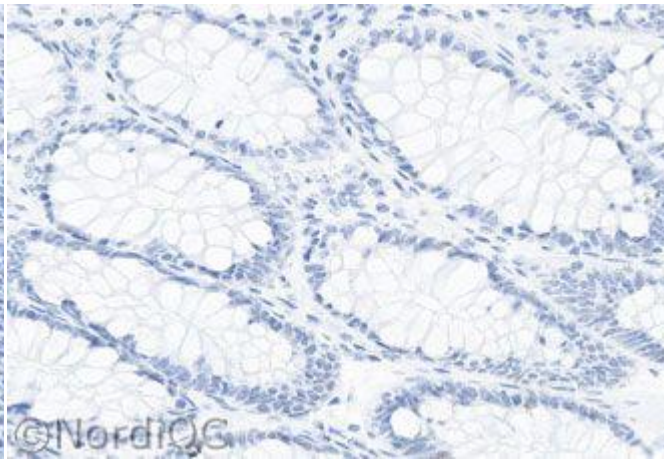

Fig. 4b
Insufficient p53 staining of the urothelial carcinoma using the same protocol as in Fig. 4a.
Dispersed neoplastic cells with a high p53 expression are demonstrated, but the excessive nuclear counter staining complicates the interpretation in the cells with a weak to moderate staining reaction – same field as in Fig. 2b.

SN/RR/LE 11-6-2013