

The slide to be stained for p53 comprised:

1. Liver, 2. Colon, 3. Adenocarcinoma (colon), 4. Serous carcinoma (ovary),
5. Endometrioid carcinoma (endometrium), 6. Fallopian tube.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a p53 staining as optimal included:

- A strong, distinct nuclear staining of $\geq 75\%$ of the neoplastic cells of the ovarian serous carcinoma and $\geq 50\%$ of the neoplastic cells of the colon adenocarcinoma.
- A strong, distinct nuclear staining in 5 – 20 % of the neoplastic cells of the endometrioid carcinoma.
- No or only a weak nuclear staining in scattered basal epithelial cells in the colon and the fallopian tube.
- No staining in the liver.

93 laboratories submitted stains. At the assessment 18 achieved optimal marks (19 %), 45 good (49 %), 27 borderline (29 %) and 3 poor marks (3 %).

The following Abs were used:

mAb clone **DO-7** (Dako; n=70, Novocastra, n=5; Ventana, n=4; NeoMarkers, n=1; Biocare Medical, n=1)

mAb clone **DO-1** (Calbiochem, n=1; Dianova, n=1; Novocastra, n=1)

mAb clone **BP53-12** (NeoMarkers, n=1; Novocastra, n=1)

mAb clone **DO-7 + BP53-12** (NeoMarkers, n=2)

mAb clone **Bp-53-11** (Ventana, n=1)

rmAb **SP5** (NeoMarkers, n=3)

pAb **NCL-p53-CM1** (Novocastra, n=1)

Optimal staining for p53 in this assessment was obtained with the mAb clone **DO-7** (14 out of 81), mAb clone **DO-1** (2 out of 3), the mAb clone **BP53-12** (1 out of 2) and the mAb cocktail **DO-7 + BP53-12** (1 out of 2).

DO-7: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) with Tris-EDTA/EGTA pH 9 or Cell Conditioning1 (BenchMark, Ventana) as HIER buffer. The mAb was typically diluted in the range of 1:100 – 1:1,000 depending on the total sensitivity of the protocol employed, or as a Ready-To-Use (RTU) product. Using these protocol settings 34 out of 44 (77 %) laboratories produced a sufficient (optimal or good) staining.

DO-1: The protocols giving an optimal result were all based on HIER using Citrate pH 6 as HIER buffer. The Ab was diluted in the range of 1:30 – 1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings 3 out of 3 (100 %) laboratories produced a sufficient staining.

BP53-12: The protocol giving an optimal result was based HIER using Citrate pH 6 as HIER buffer. The Ab was diluted 1:50.

DO-7 + BP53-12: The protocol giving an optimal result was based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Vision Biosystems) as HIER buffer. The Ab was diluted 1:200. Using these settings 1 out of 2 laboratories produced a sufficient staining.

The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Too high concentration of the primary antibody
- Less successful primary antibody

The prevalent feature of an insufficient staining was a weak/false negative staining, which was observed in 20 out of the 30 insufficient stains. The majority of the laboratories was capable to demonstrate p53 in the ovarian serous carcinoma, whereas the reaction in the colon adenocarcinoma and endometrial carcinoma was too weak or false negative.

However, also a false positive nuclear staining was observed in 10 out of the 30 insufficient stains, and was typically characterized by a moderate reaction in virtually all the epithelial cells of the fallopian tube and the majority of the benign epithelial cells of the colon.



The high proportion of both false negative and false positive stains for p53 indicates that the immunohistochemical protocol for p53 has to be carefully calibrated to be used as a diagnostic tool and that the identification of a reproducible positive and negative control is crucial to validate the threshold of the protocol for p53.

In this assessment colon adenocarcinoma and normal colon was the most reliable control, in which only scattered basal epithelial cells in the mucosa of the normal colon should display a weak reaction, while the majority of the neoplastic cells of the colon adenocarcinoma should display a strong nuclear staining.

Only antibodies raised to both wild type and mutant p53 gave sufficient results, while all 3 laboratories using the mAb clone SP5 raised to wild type p53 achieved an insufficient mark showing a too weak or false negative staining.

As no normal tissue is appropriate for control, the laboratory should use a multitissue block including tumour with varying expression of p53.

Conclusion

The mAb clones **DO-7**, **DO-1**, **BP53-12** and the mAb cocktail **DO-7 + BP53-12** are all useful for the demonstration of p53. HIER is mandatory to obtain an optimal result. Concentration of the primary Ab should be carefully calibrated.

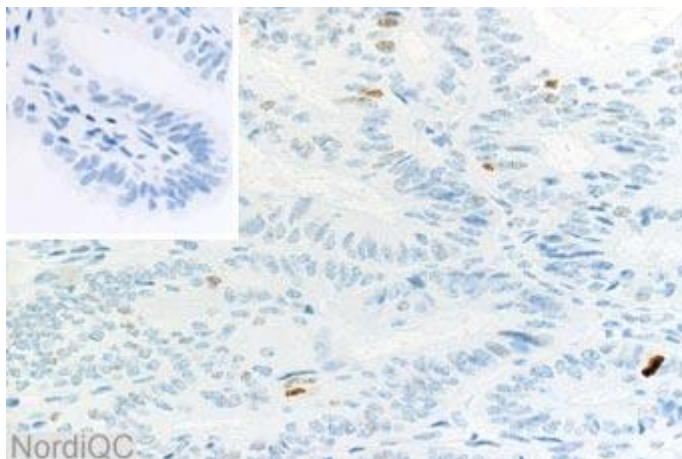


Fig. 1a
Optimal staining for p53 of the endometrioid carcinoma with clone DO7 after HIER. Only scattered tumour cells show a distinct nuclear reaction.
Insert: Same protocol and staining in the fallopian tube showing no reaction in the epithelial cells.

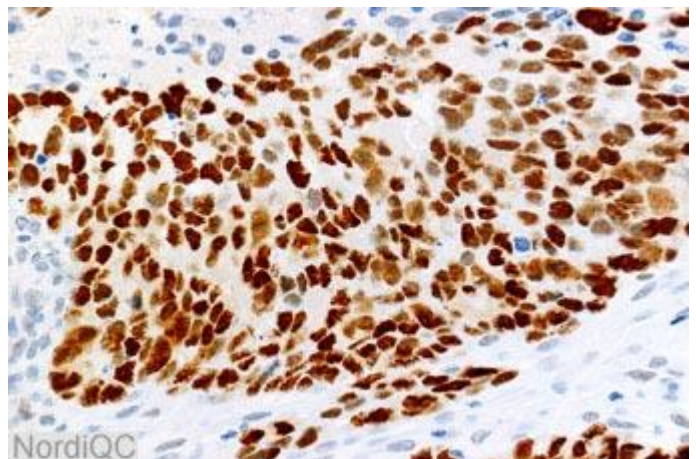


Fig. 1b
Optimal staining for p53 of the serous carcinoma. Virtually all the neoplastic cells show a strong and distinct reaction. Same protocol as in Fig. 1a.

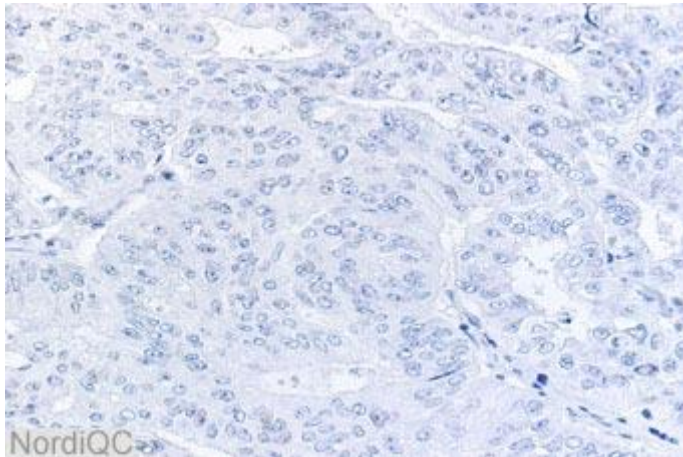


Fig. 2a
Insufficient staining for p53 of the endometrioid carcinoma. All the tumour cells are negative. The Ab was used in a too low concentration.

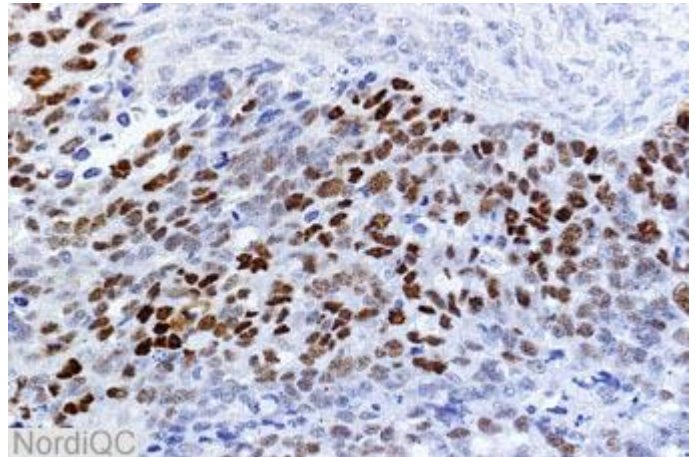


Fig. 2b
Staining for p53 of the serous carcinoma using an insufficient protocol (same protocol as in Fig. 2a). The tumour cells are stained heterogenously.

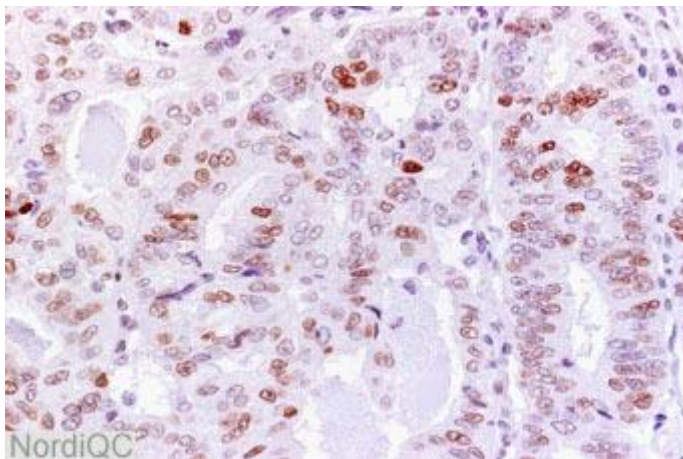


Fig. 3a
Insufficient staining for p53 of the endometrioid carcinoma. Almost all the tumour cell nuclei show a distinct staining reaction. The Ab was used in a too high concentration. Also compare with Fig. 3b.

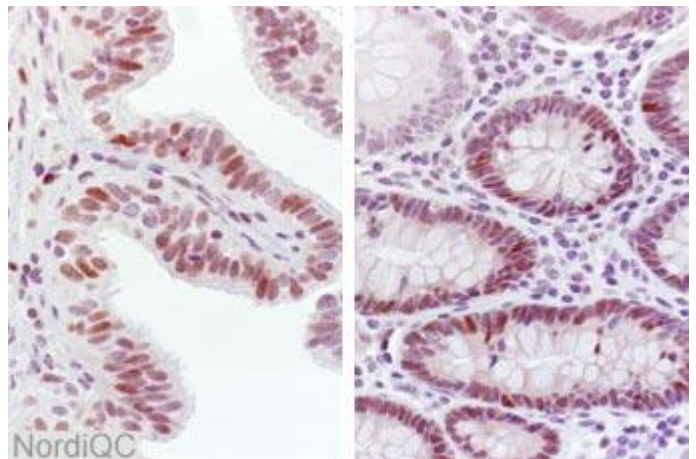


Fig. 3b
Insufficient staining for p53 of the fallopian tube and appendix, same protocol as in Fig. 3a.
Left: Both the epithelial and stromal cell nuclei show a distinct nuclear reaction.
Right: The nuclei of the enterocytes show a distinct reaction.

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