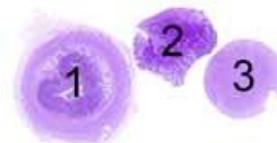


The slide to be stained for MSH2 comprised:

1: Appendix, 2: Colon adenocarcinoma with loss of MLH1 protein, 3: Colon adenocarcinoma with loss of MSH2 protein. All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing an MSH2 staining as optimal included: A strong and distinct nuclear staining of virtually all cells (enterocytes, lymphocytes, smooth muscle cells etc.) in the appendix and the two colon specimens including the adenocarcinoma in specimen 2, while the adenocarcinoma in specimen 3 should be negative. A weak cytoplasmic reaction was accepted.

26 laboratories participated in the assessment. At the assessment 7 achieved optimal (27 %), 4 good (16 %), 14 borderline (54 %) and 1 (4 %) poor marks.

The following antibody clones were used:

mAb clone **25D12** (Novocastra, n=10)
mAb clone **FE11** (Oncogene, n=4; Biocare, n=2; Calbiochem, n=1)
mAb clone **G219-1129** (BD Pharmingen, n=3; Cell Margue, n=2)
mAb clone **27** (BD Pharmingen/Transduction, n=4)

In this assessment optimal staining could be obtained with clone FE11 (3 out of 7 were optimal), clone 27 (2 out of 4) and clone 25D12 (2 out of 10), in all cases with HIER.

The range of dilutions, heating buffers and devices for optimal results were as follows:

Clone FE11 (1:150-1:600): Tris-EDTA/EGTA pH 9 (2 out of 3 were optimal) and Borg Decloaker pH 9,5 (Biocare) (1 out of 1); microwave oven (2 out of 4 were optimal) and pressure cooker (1 out of 1).

Clone 27 (1:600-1:800): EDTA pH 8 (2 out of 3 were optimal); MWO (2 out of 4).

Clone 25D12 (1:100-1:200): Tris-EDTA/EGTA pH 9 (2 out of 9 were optimal); pressure cooker (2 out of 3) while optimal results were not seen with microwave oven (0 out of 6). With clone 25D12, the staining intensity and proportion of positive cells in optimal stains were lower than with clones FE11 and 27 and at the same time giving a slight cytoplasmic reaction (fig. 3a.).

The most frequent causes of insufficient staining were:

- Too diluted primary antibody
- Insufficient HIER.

The prevalent feature of an insufficient staining was a too weak or negative staining of the majority of the cells that were expected to stain. As the identification of an MLH1 loss is characterized by a negative immunoreaction of the neoplastic cells, it is of decisive importance that the benign cells can be demonstrated and thus serves as internal positive control.

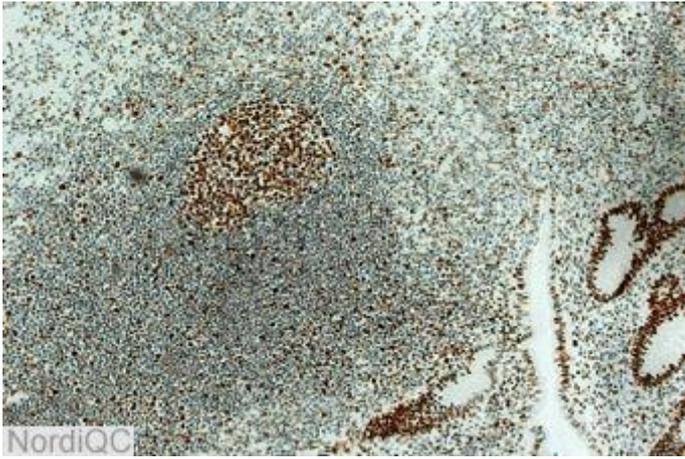


Fig. 1a
Optimal staining for MSH2 in the appendix. Almost all cells show a moderate to strong nuclear staining.

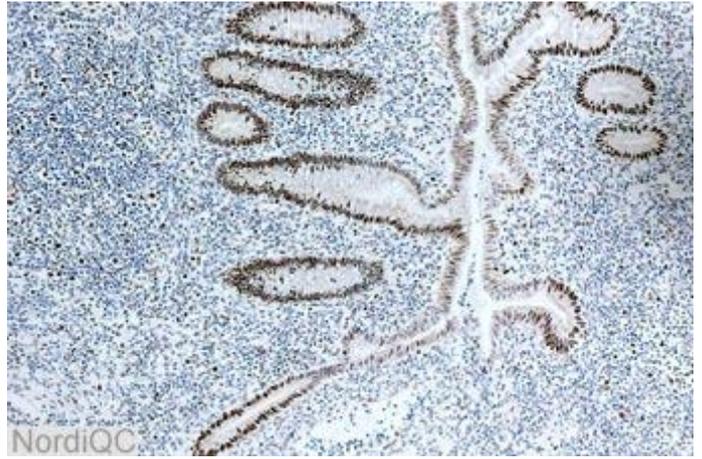


Fig. 1b.
Insufficient staining for MSH2 in the appendix. Only the enterocytes show a distinct staining, whereas most stromal cells are negative.

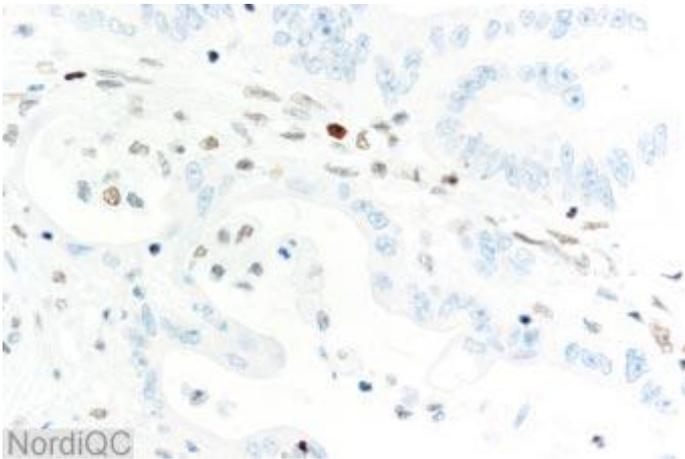


Fig. 2a
Optimal staining for MSH2 in the colon adenocarcinoma with loss of MSH2 protein. The neoplastic cells are negative and the stromal cells show a positive nuclear reaction.

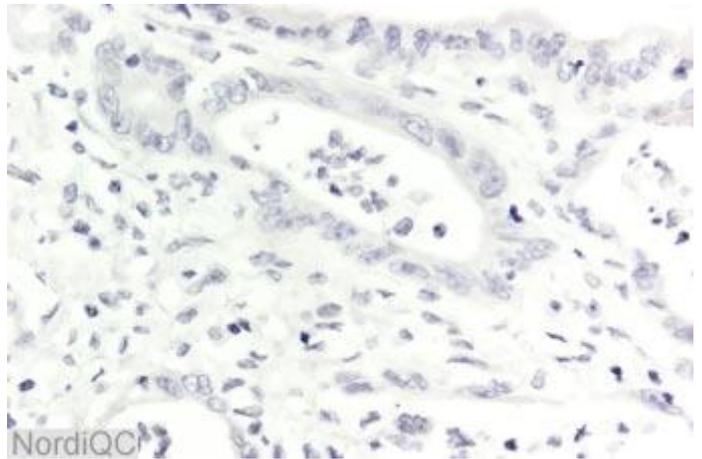


Fig. 2b
Insufficient staining for MSH2 in the colon adenocarcinoma with loss of MSH2 protein. Both the neoplastic and stromal cells are negative.

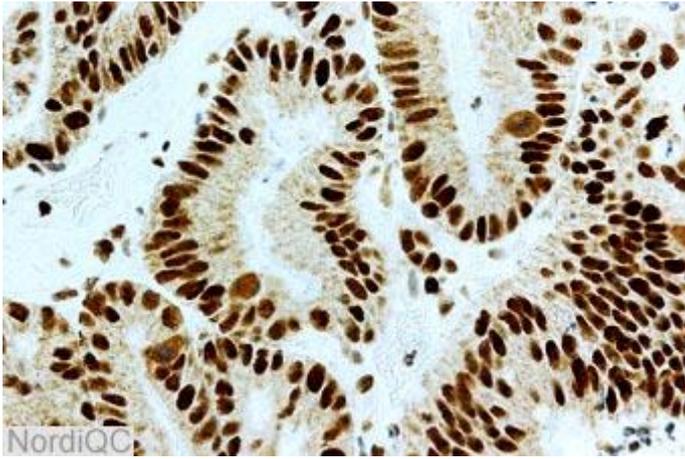


Fig. 3a
Optimal staining for MSH2 in the colon adenocarcinoma with loss of MLH1 protein using the mAb clone 25D12. The nuclei show a strong and distinct nuclear reaction but also a weak to moderate cytoplasmic reaction. This reaction pattern was observed in otherwise optimal stains using clone 25D12.

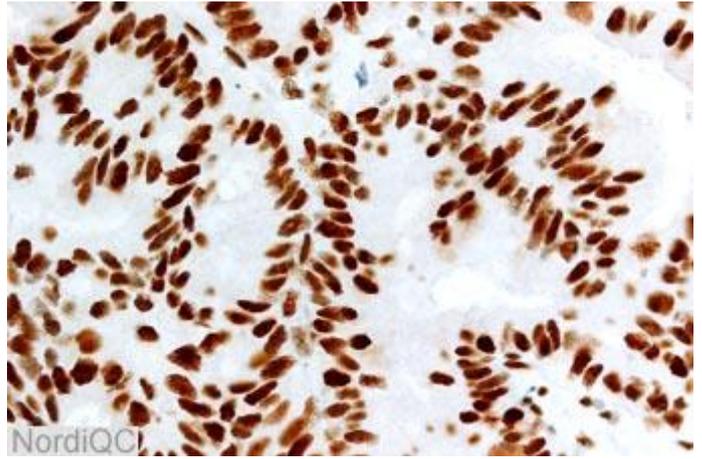


Fig. 3b
Optimal staining for MSH2 in the colon adenocarcinoma with loss of MLH1 protein using the mAb clone FE11. The nuclei show a strong and distinct nuclear reaction without cytoplasmic reaction. Compare with fig. 3a.

SN/MV/LE 4-4-2005