

Assessment Run 13 2005

Mismatch repair protein MLH1

The slide to be stained for MLH1 comprised:

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



Criteria for assessing an MLH1 staining as optimal included: A strong and distinct nuclear staining of virtually all benign cells (enterocytes, lymphocytes, smooth muscle cells etc.) in the appendix and the two colon specimens. The neoplastic cells of the adenocarcinoma with loss of MLH1 (specimen 2) should be negative, while the neoplastic cells of the adenocarcinoma with loss of MSH2 should be positive (specimen 3). A weak cytoplasmic reaction was accepted.

25 laboratories participated in the assessment. At the assessment 8 achieved optimal (32 %), 10 good (40 %), 5 borderline (20 %) and 2 (8 %) poor marks.

The following antibody clones were used:

mAb clone **G168-15** (BD Pharmingen, n=20; Biocare, n=2)

mAb clone **168-728** (Cell Margue, n=3).

In this assessment optimal staining could only be obtained with the mAb clone G168-15 (8 out of 22 were optimal). The optimal staining was based on HIER in all cases with heating buffers and devices as follows: Tris-EDTA/EGTA pH 9 (6 out of 13 were optimal), EDTA pH 8 (1 out of 4) or Borg Decloaker pH 9,5 (Biocare) (1 out of 1); MWO (5 out of 18 were optimal) or pressure cooker (3 out of 4). Clone G168-15 was typically used in dilution of 1:25 – 1:200.

The basal enterocytes and germinal centre cells were demonstrated in most protocols, but in the optimal staining all enterocytes, lymphocytes, stromal cells etc. were labelled, the latter are always included in the neoplastic lesions as an internal control. The colon adenocarcinoma with the MLH1 loss displayed a slight variation in the staining characteristics: in some stains the neoplastic cells were totally negative while in other stains, a staining reaction along the nuclear membranes was observed in some cells. This may be the result of a highly sensitive protocol (or slight over staining) and should not be interpreted as positive.

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.

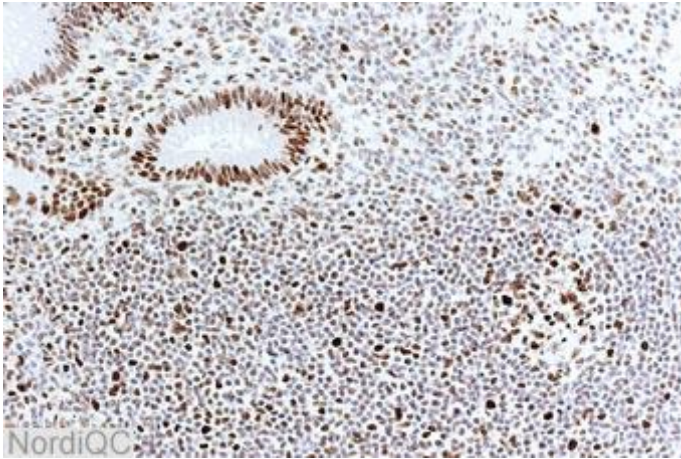


Fig. 1a
Optimal staining for MLH1 in the appendix. Almost all cells show a moderate to strong nuclear staining.

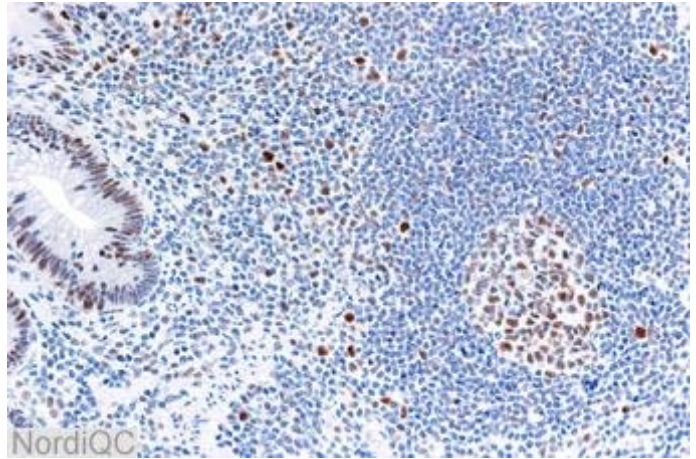


Fig. 1b
Insufficient staining for MLH1 in the appendix. Only the germinal center cells show a distinct staining, whereas most stromal cells are negative.

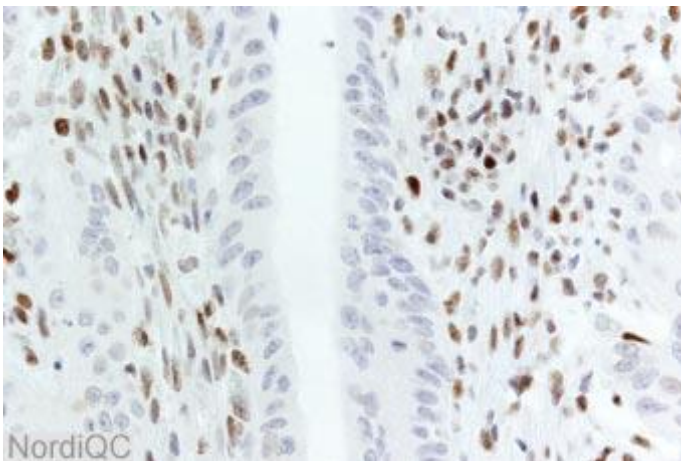


Fig. 2a
Optimal staining for MLH1 in the colon adenocarcinoma with loss of MLH1 protein. The neoplastic cells are negative and the stromal cells show a positive nuclear reaction.

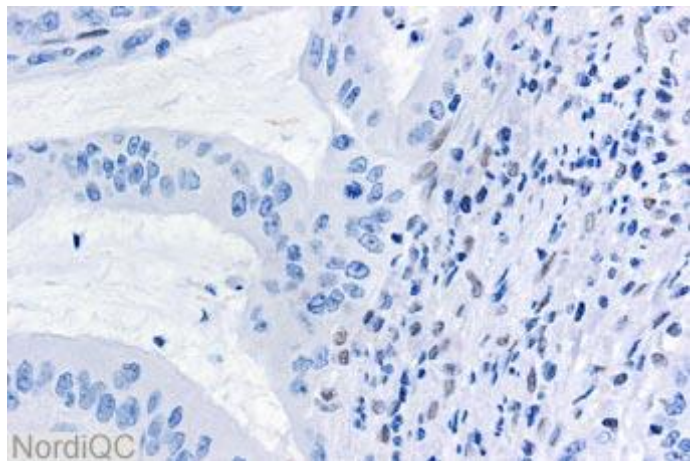


Fig. 2b
Insufficient staining for MLH1 in the colon adenocarcinoma with loss of MLH1 protein. Both the neoplastic cells and the majority of the stromal cells are negative.

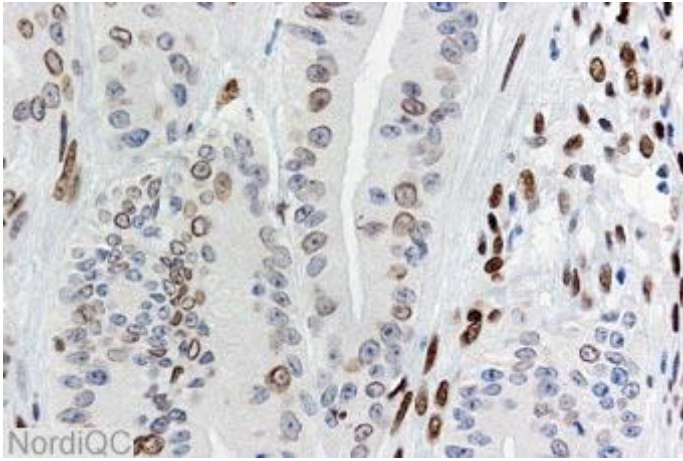


Fig. 3a
Good staining for MLH1 in the colon adenocarcinoma with loss of MLH1 protein. The stromal cells (right part of the picture) are strongly stained. In the tumour cells a staining reaction along the nuclear membranes (center and left). This must be interpreted as negative, i.e. loss of protein.

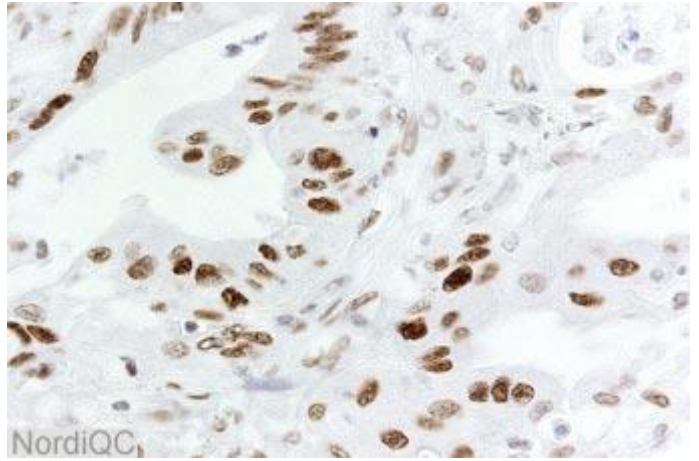


Fig. 3b
Optimal staining for MLH1 in the colon adenocarcinoma with loss of MSH2 protein. The nuclei show a more homogeneous staining without the accentuation at the nuclear membranes compared as shown in fig. 3a.

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