

The slide to be stained for Mismatch repair protein MSH2 (MSH2) comprised:  
 1. Tonsil, 2. Colon adenocarcinoma with normal expression of MSH2 protein, 3-4.  
 Colon adenocarcinoma with loss of MSH2 protein.  
 All tissues were fixed in 10% neutral buffered formalin.



Criteria for assessing a MSH2 staining as optimal included:

- A strong and distinct nuclear staining of virtually all the cells in the tonsil and the colon adenocarcinoma no. 2 – including epithelial cells, stromal cells, lymphocytes, muscle cells etc.
- A negative reaction in the neoplastic cells of the colon adenocarcinomas no. 3 & 4, while a distinct nuclear reaction should be seen in all other cells. A weak cytoplasmic reaction was accepted.

51 laboratories participated in the assessment. At the assessment 10 achieved optimal (20 %), 27 good (53 %), 7 borderline (14 %) and 7 (14 %) poor marks.

The following 5 antibody clones were used:

mAb clone **G219-1129** (BD Pharmingen, n=10; Ventana, n=7; Cell Margue, n=2; BioGenex, n=1)  
 mAb clone **FE11** (Calbiochem/Oncogene, n=7; Biocare, n=7; Zytomed, n=1)  
 mAb clone **25D12** (Novocastra, n=8)  
 mAb clone **27** (BD Pharmingen/Transduction, n=7)  
 mAb clone **GB12** (Calbiochem, n=1)

Optimal staining for MSH2 in this assessment was obtained with the mAbs clones **25D12**, **G219-1129**, **27** and **FE11** as follows:

**25D12:** 3 out of 8 laboratories (38%) produced an optimal stain. Sufficient stains (optimal or good) were seen in 7 cases (88%). The optimal protocols were all based on heat induced epitope retrieval (HIER) using Tris-EDTA/EGTA pH 9 and the Ab was diluted in the range of 1:10 - 1:60 depending on the total sensitivity of the protocol employed.

**G219-1129:** 3 out of 20 laboratories (15%) produced an optimal stain. Sufficient stains were seen in 14 cases (70%). The optimal protocols were all based on HIER using either Tris-EDTA/EGTA pH 9, EDTA/EGTA pH 8 or Cell Conditioning 1 (CC1, Ventana). The Ab could both be applied as a Ready To Use (RTU) product from Ventana (used by 2 out of 3) and as a concentrate diluted 1:200.

**27:** 2 out of 7 laboratories (29%) produced an optimal stain. Sufficient stains were seen in 6 cases (86%). The optimal protocols were both based on HIER using Tris-EDTA/EGTA pH 9. The Ab was diluted in the range of 1:800 - 1:1000.

**FE11:** 2 out of 15 laboratories (13%) produced an optimal stain. Sufficient stains were seen in 10 cases (67%). The optimal protocols were based on HIER using Tris-EDTA/EGTA pH 9. The Ab was used in in the range of 1:25 to 1:400 depending on the total sensitivity of the protocol employed.

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 false negative staining of the majority of the cells that were expected to stain (Fig. 2a - Fig. 2d). As the identification of an MSH2 loss is characterized by a negative immunoreaction of the neoplastic cells, it is of decisive importance that the benign cells can be demonstrated, thus serving as internal positive control. Stromal cells of the tumours and normal enterocytes should be strongly positive. Lymphatic tissue as in the appendix or tonsil are appropriate as positive control. The optimal protocols were all able to give a strong staining, not only in the most active and proliferating cells in the germinal centres but also in the dormant lymphocytes in the mantle zone (Figs. 3a-b).

In concordance with the previous assessment of MSH2, run 13 2005, the mAb clones FE11, 27 and 25D12 could all be used to obtain an optimal staining. However, it was observed that the latter and the mAb clone G219-1129 also gave a weak to moderate cytoplasmic reaction and labelled a reduced proportion of cells expected to stain for MSH2. The overall pass rate and proportion of sufficient stains was 73% (compared to 43% in previous run). Many factors may contribute to the improvement and it can be difficult to find specific explanations for the improvement as many laboratories have changed their entire system and the number of participants almost doubled.

### Conclusion

The mAbs clones **25D12**, **G219-1129**, **27\*** and **FE11** are all useful for the demonstration of MSH2. All clones require efficient HIER (preferable in an alkaline buffer. The concentration of the primary Ab and the immunohistochemical protocol should be carefully calibrated, so all the lymphocytes in the tonsil show a distinct nuclear reaction.

\* withdrawn from the market 2007.

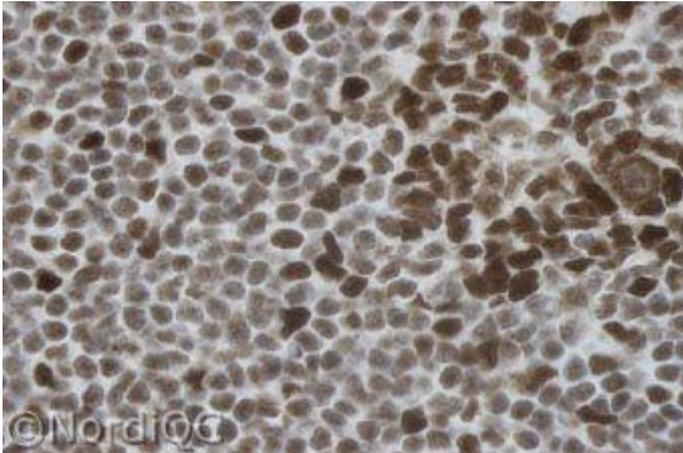


Fig. 1a  
Optimal staining for MSH2 using the mAb clone FE11 of the tonsil. A strong nuclear staining is seen in all the lymphocytes. The reaction is strongest in the germinal centre cells, but the resting cells in the mantle zones are also demonstrated.

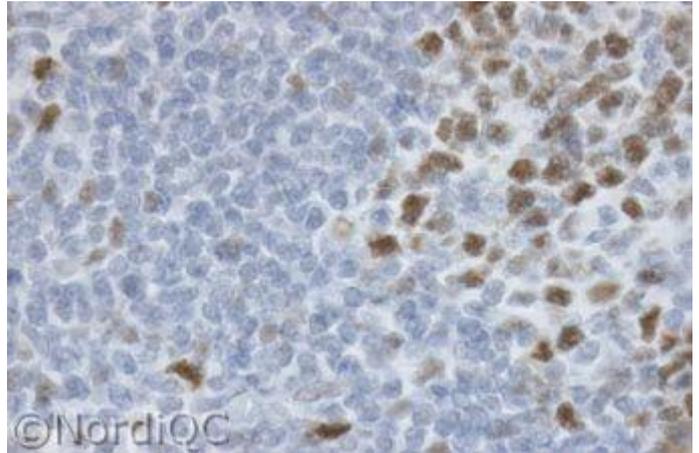


Fig. 1b  
Insufficient staining for MSH2 using the mAb clone FE11 of the tonsil. A weak to moderate nuclear staining is seen in the germinal centre, while only a few lymphocytes in the mantle zone are positive. Also compare with Fig 2b and 3b – same protocol.

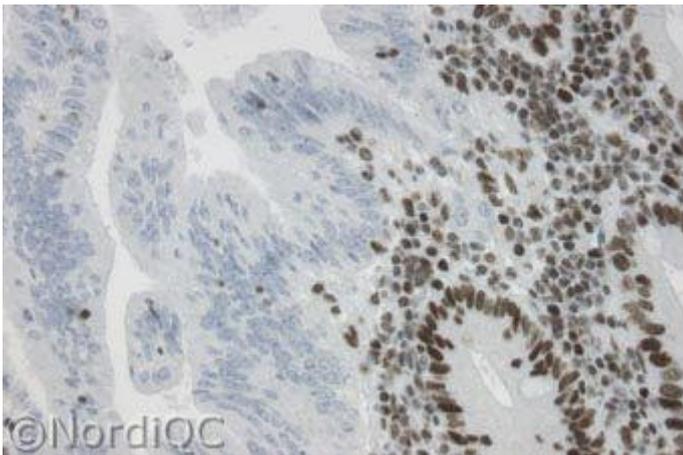


Fig. 2a  
Optimal staining for MSH2 of the colon adenocarcinoma no 3 with loss of MSH2 protein. A strong nuclear staining is seen the normal enterocytes and the intraepithelial T-cells, while the nuclei of the neoplastic cells are negative. Same protocol as in Fig. 1a.

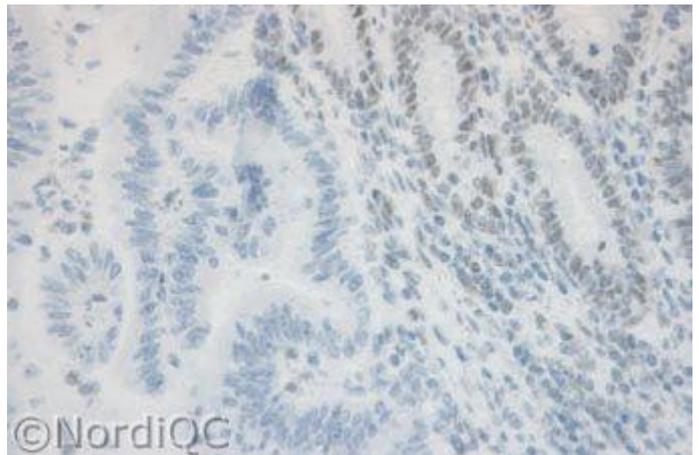
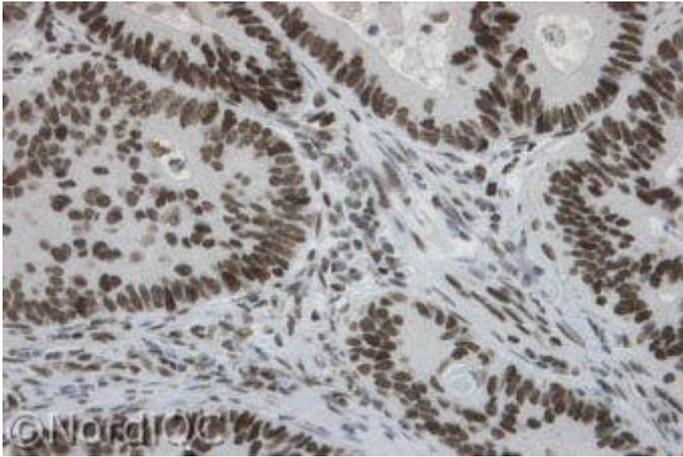
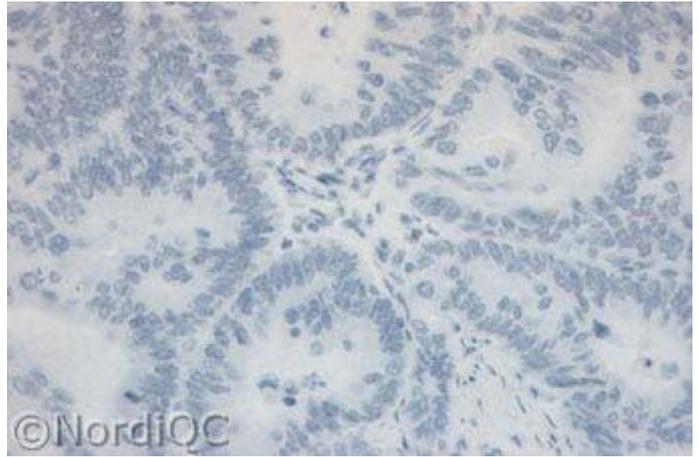


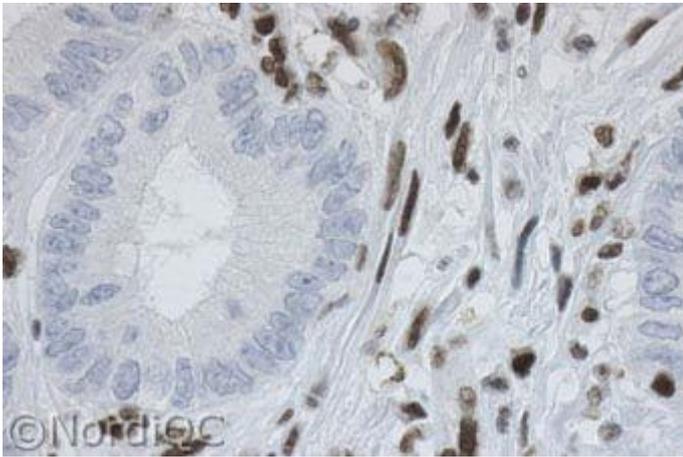
Fig. 2b  
Insufficient staining for MSH2 using same protocol as in Fig. 1b. Only a faint nuclear staining is seen in the normal enterocytes and most stromal cells are totally negative, which complicates the interpretation of a loss of the MSH2 protein in the tumour.



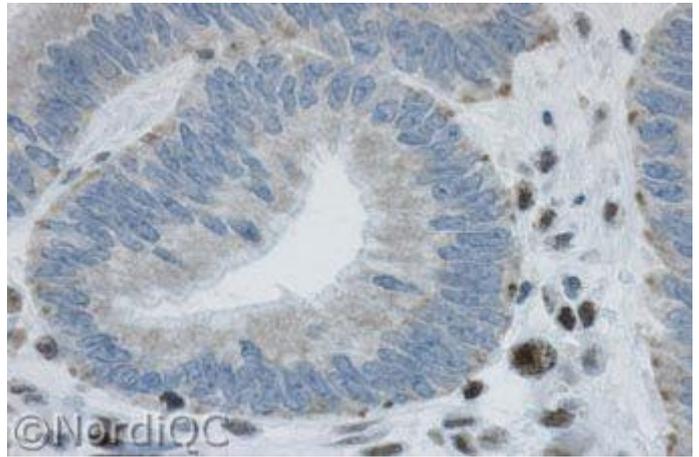
**Fig. 3a**  
Optimal staining for MSH2 of the adenocarcinoma without loss of MSH2 protein using same protocol as in Fig 1a & 2a. A strong nuclear staining is seen in all the neoplastic cells and virtually in all stromal cells.



**Fig. 3b**  
Insufficient staining for MSH2 of the adenocarcinoma without loss of MSH2 protein using same protocol as in Fig 1b & 2b. A false negative staining is seen in the neoplastic cells. All stromal cells are negative too.



**Fig. 4a**  
Optimal staining for MSH2 of a colon adenocarcinoma with loss of MSH2 protein. A strong nuclear staining is seen the stromal cells, while the neoplastic cells are negative. This pattern was typically seen using the mAb clones 27 and FE11. Compare with Fig 4b using other clones.



**Fig. 4b**  
Optimal staining for MSH2 of a colon adenocarcinoma with loss of MSH2 protein. A moderate to strong nuclear staining is seen the stromal cells, while the neoplastic cells show a negative nuclear reaction, but also a weak cytoplasmic reaction. This pattern was typically seen using the mAb clones 25D12 and G219-1129.

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