

Assessment Run 40 2014

Mismatch repair protein MLH1 (MLH1)

[Recommended MLH1 protocols](#)

[Recommended MLH1 control tissue](#)

Materials

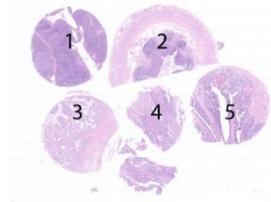
The slide to be stained for **MLH1** comprised:

1. Tonsil, 2. Appendix, 3 & 4. Colon adenocarcinoma with loss of MLH1 expression, 5. Colon adenocarcinoma with normal MLH1 expression.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing MLH1 staining as optimal were:

- An at least weak to moderate distinct nuclear staining reaction of virtually all cells in the appendix.
- An at least weak to moderate distinct nuclear staining reaction of virtually all mantle zone B-cells and a moderate to strong nuclear staining reaction of the germinal centre B-cells.
- A moderate to strong nuclear staining in virtually all neoplastic cells of the colon adenocarcinoma no. 5.
- No nuclear staining reaction of the neoplastic cells of the colon adenocarcinomas no. 3 and 4, but a distinct nuclear staining reaction in the vast majority of other cells (stromal cells, lymphocytes etc).
- A weak cytoplasmic staining reaction was accepted.



Results

142 laboratories participated in this assessment. 103 (73%) achieved a sufficient mark (optimal or good). Antibodies (Abs) used and assessment marks are summarized in table 1 (see page 2).

The most frequent causes of insufficient staining reaction were:

- Less successful primary Ab
- Use of low sensitive detection systems
- Too low concentration of the primary Ab
- Too high concentration of the primary Ab

Performance history

This was the 3rd NordiQC assessment of MLH1. A significant increase in the pass rate was seen compared to the previous run 30, 2010.

Table 2. **Proportion of sufficient results for MLH1 in the three NordiQC runs performed**

	Run 13 2005	Run 30 2010	Run 40 2014
Participants, n=	25	85	142
Sufficient results	72%	57%	73%

Conclusion

The mAb clones **ES05**, **G168-15** and **M1** could all be used to obtain an optimal staining for MLH1. In this assessment, the mAb clone ES05 was most successful, both as concentrate and as RTU format (Dako and Leica).

Irrespective of the clone applied, HIER in an alkaline buffer and use of a sensitive and specific 3-step polymer/multimer based detection system gave the highest proportion of optimal results.

The concentration of the primary antibody must be carefully calibrated.

Tonsil is a recommendable positive tissue control for MLH1: Mantle zone B-cells must show an at least weak to moderate nuclear staining reaction, while a moderate to strong nuclear staining reaction must be seen in proliferating germinal centre B-cells. Tumour tissue, e.g. colon adenocarcinoma with loss of MLH1 expression must be used as negative tissue control, in which no nuclear staining reaction in the neoplastic cells should be seen.

Table 1. **Antibodies and assessment marks for MLH1, run 40.**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 4C9C7	1	Thermo/Neomarkers	0	1	0	0	-	-
mAb clone ES05	28	Leica/Novocastra	18	11	3	3	83%	90%
	7	Dako						
mAb clone G168-15	17	BD Pharmingen	6	8	8	3	56%	82%
	6	Biocare						
	2	Zytomed						
mAb clone G168-728	2	Cell Marque	0	0	2	3	-	-
	2	Zeta Corp						
	1	BioSB						
Ready-To-Use antibodies								
mAb clone ES05 IR079	27	Dako	18	5	3	1	85%	92%
mAb clone ES05 PA0610	3	Leica/Novocastra	2	1	0	0	-	-
mAb clone ES05 ZM-0154	1	Zhonggshan	0	0	1	0	-	-
mAb clone G168-15 PM220	3	Biocare	1	2	0	0	-	-
mAb clone G168-15 PDM 148	2	Diagnostic Biosystems	1	0	1	0	-	-
mAb clone G168-728 760-4264	3	Ventana/Cell Marque	0	0	3	0	-	-
mAb clone G168-728 MAD-000372QD	1	Master Diagnostica	1	0	0	0	-	-
mAb clone M1 760-4535	36	Ventana	15	13	8	0	78%	77%
Total	142		62	41	29	10	-	
Proportion			44%	29%	20%	7%	73%	

1) Proportion of sufficient stains (optimal or good),

2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of MLH1, Run 40

The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies

mAb clone **ES05**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (5/6)*, TRS pH 9 (Dako) (3/3), Cell Conditioning 1 (CC1; Ventana) (6/14), Bond Epitope Retrieval Solution 2 (BERS2; Leica) (2/5), HIER buffer Medium (Thermo) (1/1) or Tris-EDTA/EGTA pH 9 (1/4) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 27 of 30 (90%) laboratories produced a sufficient staining result (optimal or good).

* (number of optimal results/number of laboratories using this buffer)

mAb clone **G168-15**: Protocols with optimal results were all based on HIER using TRS pH 9 (Dako) (1/4), CC1 (BenchMark, Ventana) (2/7), BERS2 (Leica) (2/6) or Tris-EDTA/EGTA pH 9 (1/4) as the retrieval buffer. The mAb was typically diluted in the range of 1:25-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 9 of 11 (82%) laboratories produced a sufficient staining result.

Table 3. Proportion of optimal results for MLH1 using concentrated antibodies on the 3 main IHC systems*

Concentrated antibodies	Dako		Ventana		Leica	
	Autostainer Link / Classic	TRS pH 6.1	BenchMark XT / Ultra	CC2 pH 6.0	Bond III / Max	ER1 pH 6.0
	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 ES05	6/7** (86%)	-	6/14 (43%)	-	2/5 (40%)	-
mAb clone G168-15	2/4	-	2/6 (33%)	-	2/3	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.

** (number of optimal results/number of laboratories using this buffer)

Ready-To-Use antibodies and corresponding systems

mAb clone **ES05**, product no. IR079, Dako, Autostainer+/Autostainer Link:

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 20 min at 95-97°C) and 20-30 min incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 22 of 24 (92%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **ES05**, product no. PA0610, Leica, Bond-max/Bond-III:

Protocols with optimal results were all based on HIER using BERS2 pH 9 (Bond, Leica) (efficient heating time 20 min at 97-100°C), 20-30 min incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings 2 of 2 (100%) laboratories produced a sufficient staining result.

mAb clone **M1**, product no. 760-4535, Ventana, BenchMark XT/Ultra:

Protocols with optimal results were typically based on HIER using short, mild, standard or extended Cell Conditioning 1, 4-64 min incubation of the primary Ab and UltraView (760-500 +/- amplification kit) or OptiView (760-700 +/- amplification kit) as detection system. Using these protocol settings 27 of 35 (77%) laboratories produced a sufficient staining result.

In this assessment and in concordance with the observations in the previous assessment of MLH1 (run 30, 2010), the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction in cells expected to be demonstrated. A too weak or false negative staining reaction was seen in 59% of the insufficient results (23 of 39). The majority of the laboratories were able to demonstrate MLH1 in the cells with a high antigen expression as proliferating germinal centre B-cells in the tonsil, basal epithelial cells of the appendix and neoplastic cells in the colon adenocarcinoma with normal MLH1 expression. Demonstration of MLH1 in cells with low antigen expression as resting mantle zone B-cells, smooth muscle cells and stromal cells was more challenging and required an optimally calibrated protocol. In this context it has to be emphasized, that identification of loss of MLH1 expression in tumours is characterized by a negative nuclear staining reaction of the neoplastic cells. Consequently, it is of decisive importance that normal cells within and around the neoplastic cells show a distinct positive nuclear staining reaction, serving as reliable internal positive tissue control.

In the remaining 41% of the insufficient results (16 of 39) false positive staining reaction or poor signal-to-noise ratio was seen. This staining pattern was typically characterized by an aberrant diffuse nuclear staining reaction of the neoplastic cells of the two colon adenocarcinomas with loss of MLH1 expression and/or excessive cytoplasmic and background staining compromising the interpretation.

These staining patterns were in particular seen for the mAb clones G168-15 and G168-728.

The mAb clone ES05 was the most widely used Ab for demonstration of MLH1 and provided a high proportion of sufficient staining results both applied as concentrated format or Ready-To-Use system from Dako and Leica (see table 1). Optimal results could be obtained on all three main IHC systems from Dako, Leica and Ventana using the clone as concentrate (see table 3). Efficient HIER in an alkaline buffer in combination with a sensitive non-biotin based detection system and a titre in the range of 1:25-100 were the main protocol prerequisites for optimal results. Especially use of 3-step polymer/multimer based detection systems seemed to provide higher proportions of optimal results compared to 2-step polymer/multimer based systems.

The mAb clone G168-15 as concentrated format could also be used to obtain optimal results on the three main IHC systems provided that an optimal calibration of the primary Ab was performed. However, the mAb was frequently used too concentrated, e.g. at a titre of 1:10, giving an excessive background staining and a poor signal-to-noise ratio.

The mAb clone G168-728 as concentrate was found to be less successful, as a consistent aberrant cytoplasmic staining reaction was seen in nerves and endothelial cells and at the same time a too weak specific nuclear staining. This was also seen for the corresponding Ready-To-Use format from Ventana/Cell Marque, prod. no. 760-4264.

In this assessment, the two RTU systems from Dako and Leica based on the mAb clone ES05 provided a slightly higher pass rate and proportion of optimal results compared to in-house validated protocols using same clone as a concentrate (see table 1).

The RTU system from Ventana based on the mAb clone M1 also provided a high proportion of sufficient results. Optimal results were typically obtained by modified and laboratory validated protocol settings using UltraView +amplification or OptiView +/-amplification as detection system compared to the recommendations given in the package inserts for the RTU format.

It is difficult to conclude on the specific causes for the improvement of the pass rate obtained in this run, as many laboratories participated for the first time and many laboratories have changed their IHC systems compared to the previous run in 2010. However, the availability and extended use of high quality RTU systems for MLH1 seems to be one of the central elements. In run 30, 2010 22% of the participants (19 of 85) used RTU systems from Dako, Leica and Ventana and grouped together, a pass rate of 53% was obtained. In this run 47% (66 of 142) of the participants used a RTU system from one of these vendors and a groupa pass rate of 82% was obtained.

Controls

Tonsil was found to be a recommendable positive tissue control for MLH1. Virtually all mantle zone B-cells must show at an at least weak to moderate nuclear staining reaction, while a moderate to strong nuclear staining reaction must be seen in the proliferating germinal centre B-cells.

Colon adenocarcinoma with loss of MLH1 expression is recommended as negative tissue control for MLH1. No nuclear staining reaction should be seen in the neoplastic cells, whereas a nuclear staining reaction must be seen in stromal cells. A weak staining reaction of the nuclear membranes can be seen in scattered neoplastic cells in colon adenocarcinomas with loss of MLH1 expression.

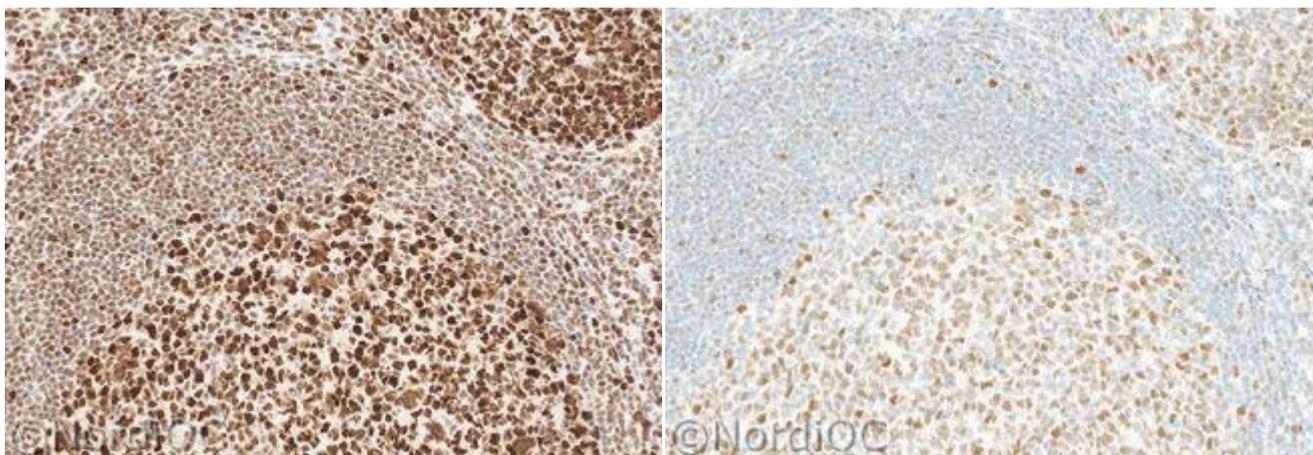


Fig. 1a

Optimal staining reaction for MLH1 of the tonsil using the mAb clone ES05, HIER in an alkaline buffer and a 3-step polymer based detection system. Virtually all the mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction.

Also compare with Figs. 2a and 3a, same protocol.

Fig. 1b

Insufficient staining reaction for MLH1 of the tonsil using the mAb clone ES05 with a protocol providing a too low sensitivity (2-step multimer based detection system and/or a too low concentration of the primary Ab) - same field as in Fig. 1a.

Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing a low level of MLH1 are virtually unstained.

Also compare with Figs. 2b and 3b, same protocol.

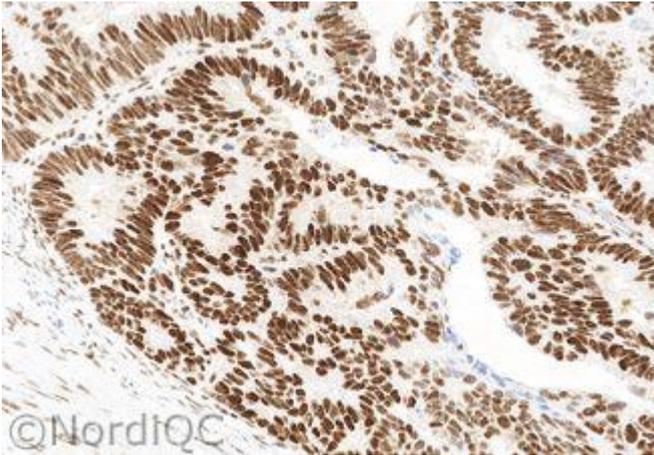


Fig. 2a
Optimal staining reaction for MLH1 of the colon adenocarcinoma tissue core no. 5 with normal MLH1 expression using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained. No background staining is seen and a distinct nuclear staining reaction in the stromal cells is seen.

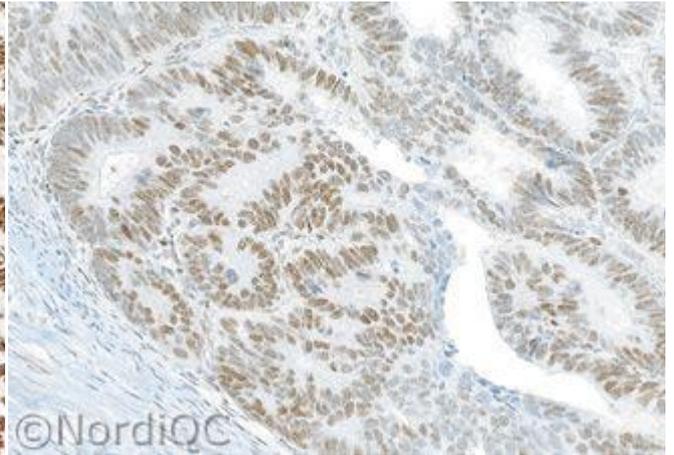


Fig. 2b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma tissue core no. 5 using same protocol as in Fig. 1b - same field as in Fig. 2a. The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result in Fig. 2, especially note the stromal cells are virtually negative. Also compare with Fig. 3b, same protocol.

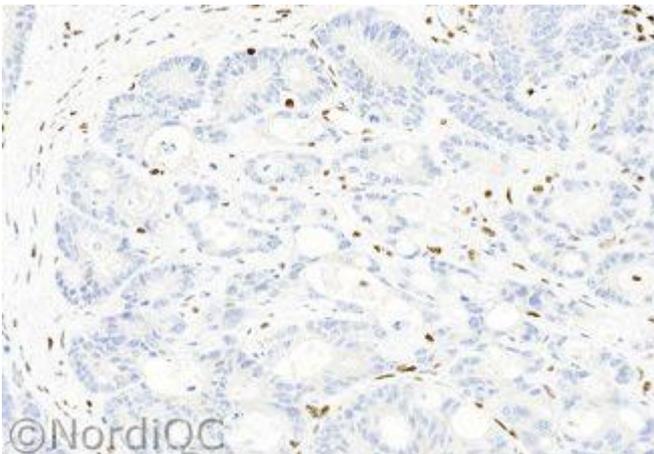


Fig. 3a
Optimal staining reaction for MLH1 of the colon adenocarcinoma no. 3 with loss of MLH1 using same protocol as in Figs. 1a & 2a. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

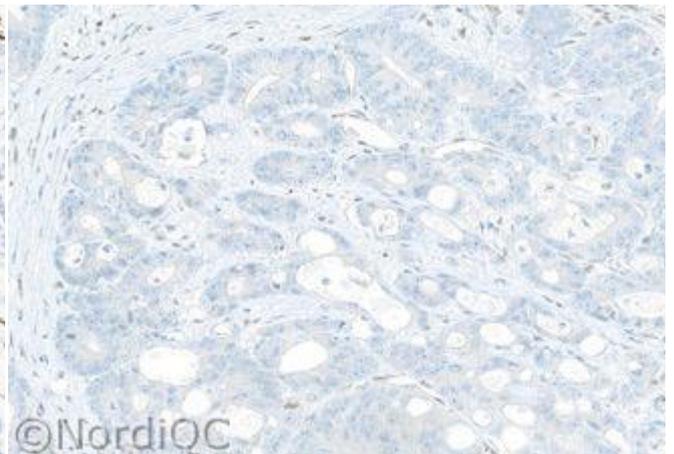


Fig. 3b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma no. 3 with loss of MLH1 using same protocol as in Figs. 1b & 2b - same field as in Fig. 3a. No staining reaction in the neoplastic cells is seen, but as also virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern can not reliably be interpreted.

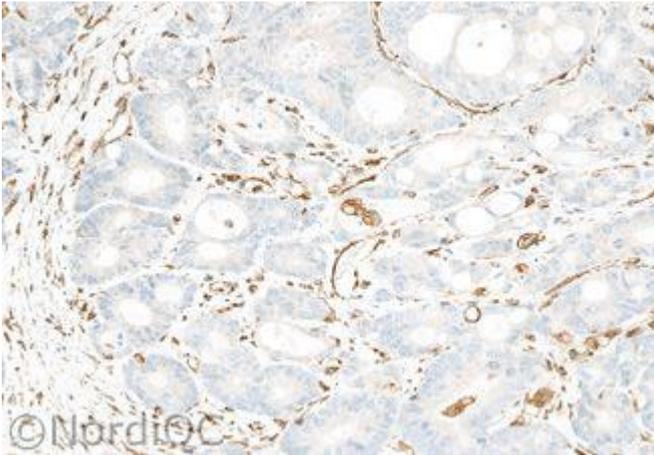


Fig. 4a
Insufficient staining reaction for MLH1 of the colon adenocarcinoma no. 3 with loss of MLH1 using the mAb clone G168-728 – same field as in Fig. 3a. No nuclear staining reaction in the neoplastic cells is seen, but an aberrant cytoplasmic staining reaction in e.g. macrophages and endothelial cells complicates the interpretation, as the nuclear staining reaction in the stromal cells cannot be identified with certainty. This staining pattern was frequently seen for the mAb clone G168-728.

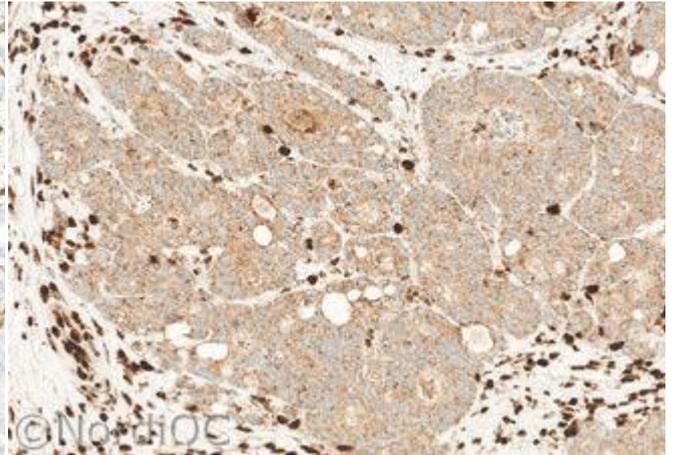


Fig. 4b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma no. 3 with loss of MLH1 using the mAb clone ESO5 – same field as in Fig. 3a. An aberrant and diffuse cytoplasmic staining reaction in the neoplastic cells is seen compromising the interpretation of a nuclear staining reaction. This staining pattern most likely was caused by a too high concentration of the primary antibody in combination with a high sensitive detection system (OptiView + amplification, Ventana).

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