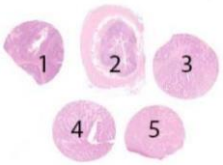
Assessment Run 67 2023

Mismatch Repair Protein MLH1 (MLH1)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for MLH1 status in colon adenocarcinomas. Loss of MLH1 function due to gene mutation or epigenetic changes is characterized by absence of nuclear expression in neoplastic cells, whereas intact nuclear MLH1 expression indicates normal MLH1 function and no gene mutations.

Material
The slide to be stained for MLH1 comprised:
1. Tonsil, 2. Appendix, 3. Colon adenocarcinoma with normal MLH1 expression,
4. & 5. Colon adenocarcinoma with loss of MLH1 expression.



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an MLH1 staining as optimal included:

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

Participation

Number of laboratories registered for MLH, run 67	370
Number of laboratories returning slides	342 (92%)

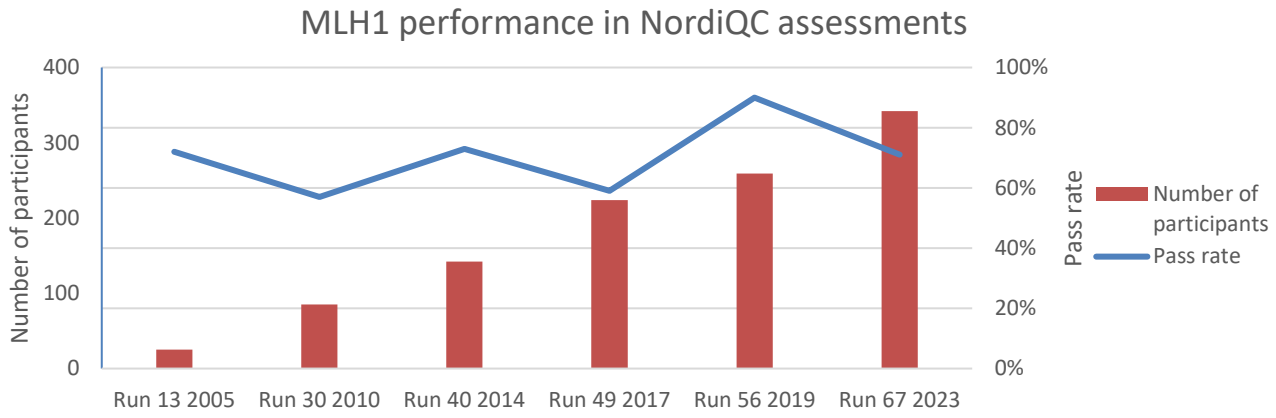
Results
At the date of assessment, 92% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

342 laboratories participated in this assessment and 71% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining were:
- Less sensitive detection systems used in combination with other low sensitivity protocol parameters e.g.
  - inefficient HIER; short time, low pH buffer
  - too low titre of primary Ab
- Unexplained technical issues

Performance history
This was the sixth NordiQC assessment of MLH1. The pass rate has decreased compared to the results obtained in the previous run (see Graph 1).

Graph 1. **Proportion of sufficient results for MLH1 in the six NordiQC runs performed**



Conclusion
The mAb clones **ES05**, **M1**, **G168-15**, **BS29**, **C12A19, GM002**, **GM011**, **IHC409** and **MX063** could all be used to obtain an optimal staining result for MLH1. Irrespective of the clone applied, sufficient Heat Induced Epitope Retrieval (HIER), use of a highly sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result. The concentrated format of the mAb clone ES05 provided an optimal result on all the main fully automated IHC platforms (Ventana/Roche, Dako/Agilent and Leica Biosystems), while the RTU systems of this clone provided a high proportion of sufficient results on their respective stainer platforms. Overall, the mAb clone ES05 (both concentrate and RTU) stained on the Leica Bond stainer platforms provided the highest pass rate of 94% and 82% optimal when used together with an alkaline HIER buffer and a 3-step detection system. In this assessment, the proportion of laboratories using an RTU format of MLH1 increased from 66% to 81% in part contributed to an RTU system for the Dako Omnis being launched after the latest MLH1 assessment run 56. In line with the previous assessment, many laboratories used the RTU IR079/IS079 (mAb clone ES05) intended for the Dako Autostainer on the Dako Omnis which provided a reduced pass rate (27%) and proportion of optimal staining results (7%) compared to the results on the intended platform (72% pass rate and 68% optimal, respectively). The RTU system based on the mAb clone M1 developed for Ventana Benchmark Ultra platform was used by 130 laboratories and most successful giving a pass rate of 90% when applied by laboratory modified protocol settings using OptiView together with OptiView Amplification Kit.

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 with loss of MLH1 (e.g. colon adenocarcinoma) expression must be used as negative tissue control, in which no nuclear staining reaction should be seen in the neoplastic cells, whereas a nuclear staining reaction must be seen in the surrounding stromal cells.

Table 1. **Antibodies and assessment marks for MLH1, Run 67**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **ES05**	20 28	Dako/Agilent Leica/Novocastra	22	14	9	3	75%	46%
mAb clone **G168-15**	7 2 1	BD Pharmingen Biocare Medical Thermo Scientific/ Epredia	3	4	2	1	70%	30%
mAb clone **BS29**	1 1	Nordic Biosite Master Diagnostica	1	1	0	0	-	-
mAb clone **GMO11**	1	PathnSitu	1	0	0	0	-	-
mAb clone **IHC409**	1	GenomeMe	1	0	0	0	-	-
mAb clone **QM003**	1	Quartett	0	0	0	1	-	-
mAb clone **ZR347**	1	Zeta Corporation	0	0	0	1	-	-
Conc total	64		28	19	11	6	73%	44%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **M1 760-5091/ 790-5091/780-7140**[3]	30	Ventana/Roche	6	13	11	-	63%	20%
mAb clone **M1 760-5091/ 790-5091/780-7140**[4]	100	Ventana/Roche	41	29	29	1	70%	41%
mAb clone **ES05 IR/IS079**[3]	12	Dako/Agilent	8	-	3	1	67%	67%
mAb clone **ES05 IR/IS079**[4]	37	Dako/Agilent	15	4	13	5	51%	41%
mAb clone **ES05 GA079**[3]	41	Dako/Agilent	27	7	7	-	83%	66%
mAb clone **ES05 GA079**[4]	17	Dako/Agilent	8	7	1	1	88%	47%
mAb clone **ES05 PA0988**[3]	4	Leica Biosystems	1	1	2	-	-	-
mAb clone **ES05 PA0988**[4]	19	Leica Biosystems	15	1	3	-	84%	79%
mAb clone **ES05 AM703**	1	BioGenex	-	-	-	1	-	-
mAb clone **BS29 MAD-000726QD**	1	Master Diagnostica	-	-	1	-	-	-
mAb clone **C12A19 CMM-0182**	1	Celnovte Biotechnology	1	-	-	-	-	-
mAb clone **DGM031**	1	Shanghai DG Diagnology Tec	-	-	1	-	-	-
mAb clone **G168-15 PM220**	1	Biocare Medical	1	-	-	-	-	-
mAb clone **G168-15 PDM148**	1	Diagnostic BioSystems	1	-	-	-	-	-
mAb clone **G168-15 BMS033**	3	Zytomed Systems	1	1	-	1	-	-
mAb clone **G168-728 285M**	2	Cell Marque	-	1	1	-	-	-
mAb clone **GM002 GT2304**	1	Gene Tech	1	-	-	-	-	-
mAb clone **GM011 8324-C010**[3]	2	Sakura Finetek	1	1	-	-	-	-
mAb clone **IHC409 unknown**	1	Zybio	1	-	-	-	-	-
mAb clone **IHC409 BFM-0166**	1	Bioin Biotechnology	1	-	-	-	-	-
mAb clone **MX063 MAB-0838**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone **521I4D5 PA242**	1	Abcarta	-	-	1	-	-	-
RTU total	278		130	65	73	10	70%	47%
Total	342		158	84	84	16		
Proportion			46%	25%	25%	5%	71%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 asessed protocols).

Detailed analysis of MLH1, Run 67
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **ES05**: Protocols with optimal results were typically based on HIER using Target Retrieval Solution (TRS, Dako/Agilent) pH 9 (3-in-1) (2/6)*, Cell Conditioning 1 (CC1, Ventana/Roche) (8/24), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (11/16) as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:75 depending on the total sensitivity of the protocol employed. Using these protocol settings, 32 of 42 (76%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **G168-15**: Protocols with optimal results were typically based on HIER using CC1 (Ventana/Roche) (3/8)* as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 7 of 8 (88%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for MLH1 for the most commonly used antibody as concentrate on the four main IHC systems***

Concentrated antibody	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark XT / Ultra**		**Leica Biosystems Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	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**	1/2**	-	1/4	-	8/23 (35%)	-	11/14 (79%)	0/1
mAb clone **G168-15**	-	-	-	-	3/8 (38%)	-	-	-

* 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 **M1**, product no. **760-5091/790-5091/780-7140**, Ventana/Roche, BenchMark GX/XT/ULTRA:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-92 min. at 95-100°C), 4-52 min. incubation of the primary Ab and OptiView (760-700) as detection system with OptiView Amplification kit (760-099 / 860-099) (incubation 4+4 min.). Using these protocol settings, 39 of 42 (93%) laboratories produced a sufficient staining result.

mAb clone **ES05**, product no. **IR/IS079**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20 min. at 95-98°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8002) as a detection system. Using these protocol settings, 14 of 19 (74%) laboratories produced a sufficient staining result (optimal or good).
23 laboratories used the RTU format off-label (deviant platforms).

mAb clone **ES05**, product no. **GA079**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (efficient heating time 30 min. at 97°C), 20-40 min. incubation of the primary Ab and EnVision FLEX+/FLEX++ (GV800/GV823/GV809/GV821) as detection system. Using these protocol settings, 45 of 53 (85%) laboratories produced a sufficient staining result.
Four laboratories used the RTU format off-label (deviant platforms).

mAb clone **ES05,** product.no. **PA0988**, Leica Biosystems, BOND III/MAX:
Protocols with optimal results were typically based on HIER in BERS2 (efficient heating time 20-30 min. at 95-100°C), 15 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 9 of 9 (100%) laboratories produced an optimal staining result.

mAb clone **GM011**, product no. **8324-C010**, Sakura FineTek, Tissue-Tek Genie Advanced:
One protocol with an optimal result was based on HIER using Tissue-Tek Genie High pH Antigen Retrieval Solution (efficient heating time 45 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek Genie Pro Detection Kit (8826-K250) as detection system.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly according to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for MLH1 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra mAb M1 **760-5091/790-5091/780-7140**	63% (19/30)	20% (6/30)	71% (67/94)	41% (39/94)
Dako AS mAb ES05 **IR/IS079**	67% (8/12)	67% (8/12)	77% (10/13)	69% (9/13)
Dako Omnis mAb ES05 **GA079**	83% (34/41)	66% (27/41)	93% (13/14)	50% (7/14)
Leica Bond III/MAX mAb ES05 **PA0988**	50% (2/4)	25% (1/4)	83% (15/18)	78% (14/18)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment of MLH1, the prevalent feature of an insufficient result was either a too weak or false negative staining reaction of cells expected to be demonstrated, which was seen in 87% (87/100) of the insufficient results. The majority of the remaining insufficient results (9/13) displayed a false positive staining reaction, a poor signal-to-noise ratio and/or an excessive background staining complicating the interpretation. Generally, most laboratories could demonstrate MLH1 in cells with high-level 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-level antigen expression as resting mantle zone B-cells, smooth muscle cells and stromal cells was more challenging and required an optimally calibrated protocol. Identification of loss of MLH1 expression in tumors 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.

19% (64 of 342) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for MLH1 with 73% (47/64) producing a sufficient result, 44% (28/64) optimal. Optimal staining results could be obtained with the mAb clones ES05, G168-15, BS29, GMO11 and IHC409 (see Table 1). The mAb clone ES05 was the most widely used concentrated Ab for demonstration of MLH1 and provided a high proportion of sufficient staining results (75%). Optimal results could be obtained on all main automated staining platforms from Ventana/Roche, Dako/Agilent and Leica Biosystems, however it was prevalent that the antibody clone performed most robustly on the Leica Bond platform providing a pass rate of 88% (15/17), 65% optimal, while the proportion of sufficient results obtained on the Ventana Benchmark platform was 64% (16/25) and all participants receiving an optimal result were using the OptiView visualization system together with the OptiView Amplification Kit. Taking into consideration all laboratories using the mAb clone ES05 as a concentrate, the average dilution factor for obtaining a sufficient result was 1:39 and respectively 1:59 for an insufficient result.

The mAb clone G168-15 as concentrated format could be used to obtain optimal results on the Ventana Benchmark Ultra platform, no participants used it on any other main staining platform. Prerequisites for an optimal staining result based on the limited data (3 laboratories) were 48-64 min. HIER in Ultra CC1 (950-224), sufficient titer of the antibody (dilution factor of 1:10 for mAb clone G168-15 produced by BD Pharmingen, 1:50-1:100 for mAb produced by Biocare Medical) and use of OptiView with OptiView Amplification Kit.

As a ready-to-use (RTU) system for all four main IHC staining platforms is available, the majority of participants opted for an RTU system, amounting to 81% (277/342) of all results.

The most widely used RTU systems for MLH1 were the Ventana/Roche 760-5091/790-5091/780-7140 products based on the mAb clone M1, used by 130 (38%) participants and resulting in an overall pass rate of 68% (89/130) and 36% (47/130) receiving an optimal mark. 23% (30/130) of laboratories used the product according to the vendor recommended protocol settings (VRPS) with OptiView visualization system which provided a pass rate of 63% (19/30), 20% (6/30) optimal. In total 94 participants modified the protocol settings for the Ventana/Roche MLH1 RTU system including changes in HIER time, primary Ab incubation time and detection system which overall slightly improved the pass rate to 71%, 41% optimal. The most successful modification was observed for the 41 participants applying OptiView Amplification Kit with incubation times of 4+4 min, in order to enhance the MLH1 signal. This raised the pass rate amongst these laboratories to 93% (38/41) and optimal rate to 73% (30/41). For the successful protocols based on OptiView with OptiView Amplification kit the mean HIER time in CC1 was 58 min. and mean Ab incubation time 24 min. (see Fig. 6a). However, meticulous care must be taken when optimizing the protocol with OptiView Amplification Kit in order to avoid an overall granular staining reaction, known to be one of the caveats of using the tyramide based amplification system which can interfere with interpretation (see Fig. 6b). In this assessment it was seen that the risk for unspecific granular staining was higher in protocols with extended incubation times for the OptiView Amplification kit reagents.
Nine participants used the mAb clone M1 based RTU system with UltraView visualization system with or without the (UltraView) Amplification Kit, none of them received an optimal mark. Although the product is only validated for the Ventana Benchmark Ultra staining platform, 7 laboratories applied it on the Ventana Benchmark GX and XT, where a sufficient result could only be obtained by using OptiView together with the OptiView Amplification Kit.

In general, it must be emphasized that modifications of vendor recommended protocol settings for the RTU systems including migration of the RTU Abs to another platform than the intended, require a meticulous validation process for the end-users. As seen in this and previous assessments, modifications can be very successful but may also generate sub-optimal or aberrant results and therefore must be carefully monitored.

The second most widely used RTU system was the recently introduced Dako/Agilent GA079 product for Dako Omnis, based on mAb clone ES05 which provided a pooled pass rate of 84% (49/58), 60% (35/58) optimal. The highest proportion of optimal results (66%) was obtained by implementing VRPS based on the use of a dual linker protocol (see Table 3). In total 41 participants used the GA079 product with VRPS and 17% (7/41) received an insufficient result due to a too weak staining, which might partly be explained by the instability of the antibody. Internal studies have shown that mAb clone ES05 is known to be more labile which was also suggested by the vendor as the onboard stability time for GA079 was significantly lower (180h) than for other Dako Omnis RTU systems however since December 2021 it has been increased to the standard 375h. This illustrates how important the use of on-slide controls is to verify the reagents used for staining patient samples.

The RTU system IR079/IS079 based on the mAb clone ES05 developed for Dako Autostainer showed an inferior pass rate compared to the last MLH1 assessment (run 56) where the overall pass rate was 87% compared to 55% in this assessment. The pooled proportion of sufficient results amongst laboratories using IR/IS079 on the Dako Autostainer was 72% (18/25), 68% (17/25) optimal, see pass rates of VRPS and LMPS separately in Table 3. Although a new product for MLH1 for Dako Omnis has been developed and is accessible, many laboratories still use the IR/IS079 RTU system on the fully automated Dako Omnis. As seen in previous assessments, optimizing the Dako Autostainer RTU antibody on other staining platforms has proven to be difficult, especially on the Dako Omnis. This type of "mismatch" provided a pass rate of 27% (4/15), with only one participant (7%) receiving an optimal mark. The most robust use of IR/IS079 on a discordant platform was with the Leica Bond, as all 4 laboratories using this platform received an optimal staining result with different protocol settings.

As mentioned above, the mAb clone ES05 seems to be very robust together with the Leica Bond staining platform and Bond Refine (DS9800) visualization system. The corresponding Leica Biosystems RTU system PA0988 showed the highest pass rate of 100% (14/14) and 93% (13/14) of optimal results when used together with HIER in alkaline BERS2 buffer instead of the recommended low pH BERS1 (Bond Epitope Retrieval Solution 1) HIER buffer. The proportion of sufficient results amongst participants using the vendor recommended HIER buffer BERS1 (AR9961) was 38% (3/8), 25% optimal and all insufficient results were characterized by a too weak staining reaction.

The sixth assessment of MLH1 provided an overall pass rate of 71% which is significantly lower compared to the previous run in 2019 resulting in a pass rate of 90%. 123 new participants took part in this assessment and the pass rate amongst them was slightly lower (64%) compared to laboratories participating in both run 56 and 67 (74%). In this assessment, the proportion of laboratories using an RTU

format of MLH1 increased from 66% to 81% as an RTU system for the Dako Omnis was launched after the last MLH1 assessment. The reduction of sufficient results was seen throughout all antibodies/IHC systems also being used in the previous run as well which can indicate that a more difficult tissue micro-array (TMA) has been circulated. However, it was observed that many participants de facto used identical protocol settings for the same antibodies/IHC systems and obtained a variety of staining intensities and proportion of cells demonstrated which also points to the technical difficulties that laboratories are facing to secure test reproducibility for MLH1. It has to be noted that IHC for MLH1 needs very sensitive protocol settings in order to ensure the visualization of cells with a low expression of MLH1, especially the intratumoral stromal cells which serve as an indispensable internal control of the patient sample and even minor fluctuations in the required level of analytical sensitivity can have a negative impact for the IHC result.

Controls

Tonsil was found to be a recommendable positive tissue control for MLH1. Virtually all 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 all 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 the surrounding stromal cells and lymphocytes.

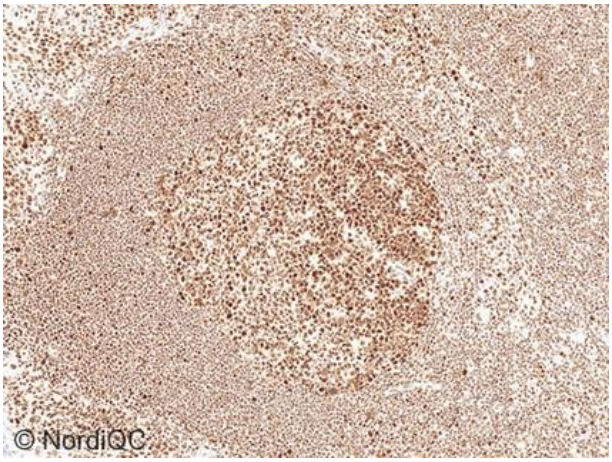


Fig. 1a
Optimal staining reaction for MLH1 of the tonsil using the mAb clone ES05 in a RTU format (PA0988) on the Leica Bond instrument using BERS2 (AR9640) buffer solution for HIER. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Also compare with Figs. 2a - 4a, same protocol.

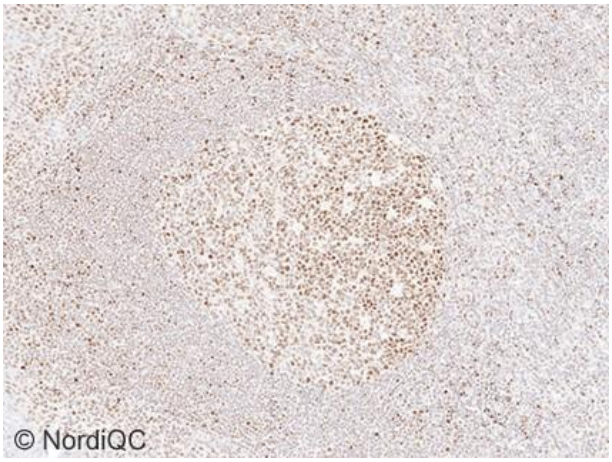


Fig. 1b
Insufficient staining reaction for MLH1 of the tonsil using the mAb clone M1 in a RTU format (790-5091) on the Ventana Benchmark Ultra instrument together with less sensitive protocol settings with too short HIER time (24 min.). Compare with Fig. 1a – same field. Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing a low level of MLH1 are virtually unstained. Also compare with Figs. 2b – 4b, same protocol.

Fig. 2a
Optimal staining reaction for MLH1 of the appendix using the same protocol as in Fig. 1a. In the mucosa-associated lymphoid tissue, virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells and virtually all crypt columnar epithelial cells show a strong nuclear staining reaction.

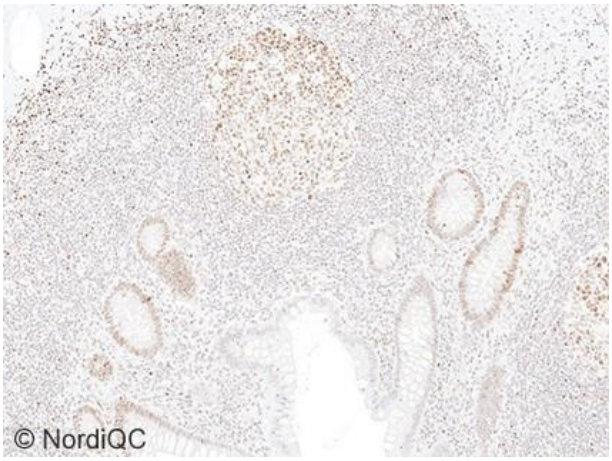


Fig. 2b
Insufficient staining reaction for MLH1 of the appendix using the same protocol as in Fig. 1b. Only the crypt epithelial cells and the germinal centre B-cells are demonstrated, while the mantle zone B-cells and most other cells expressing a low level of MLH1 are virtually unstained.

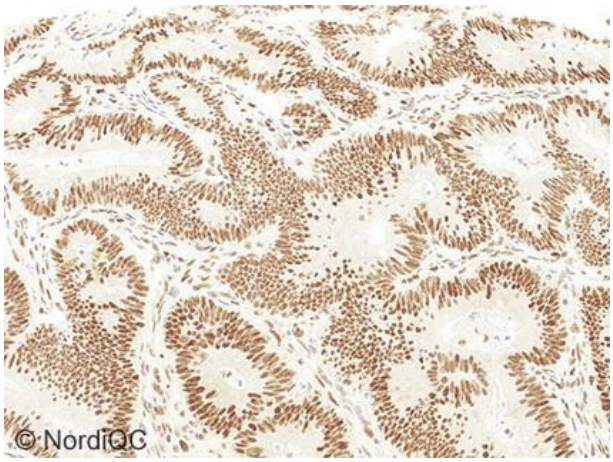


Fig. 3a
Optimal staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 3, with normal MLH1 expression using same protocol as in Figs. 1a and 2a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. Stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

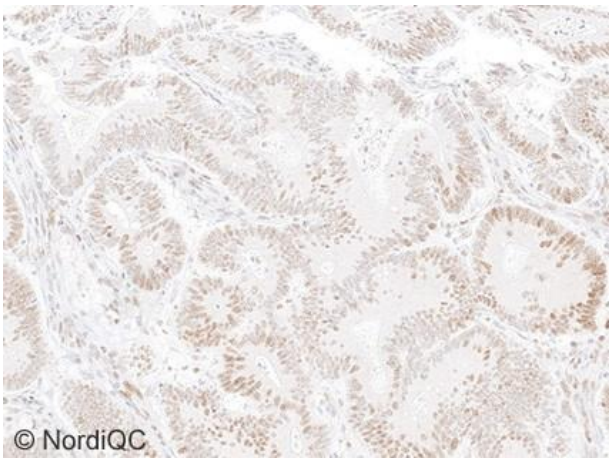


Fig. 3b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 3, using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. The proportion of positive neoplastic cells and the intensity of the staining reaction are reduced compared to the result in Fig. 3a. Only a weak staining reaction is seen in a significantly reduced number of stromal cells. Also compare with Fig. 4b, same protocol.

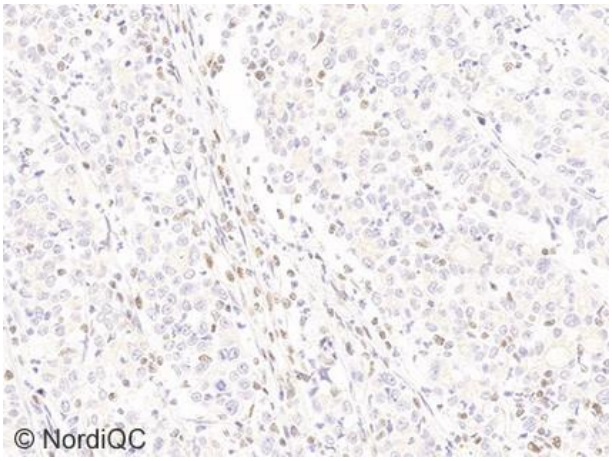


Fig. 4a
Optimal staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 5, with loss of MLH1 using same protocol as in Figs. 1a-3a. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

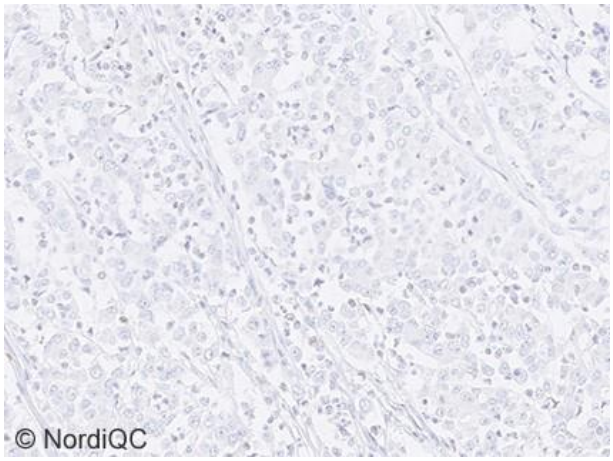


Fig. 4b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 5, with loss of MLH1 using same protocol as in Figs. 1b-3b, same field as in Fig. 4a. No staining reaction in the neoplastic cells is seen, but as virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern cannot reliably be interpreted.

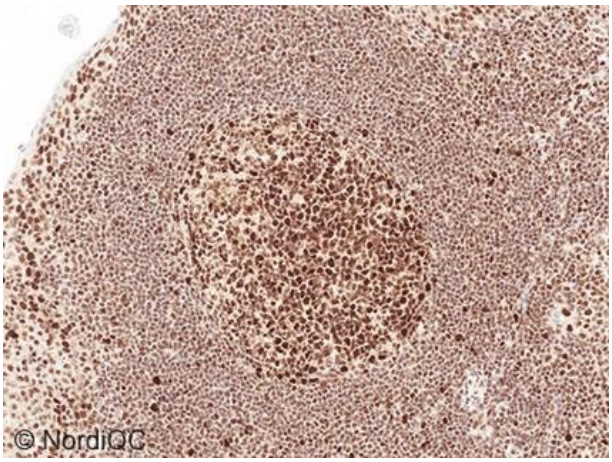


Fig. 5a
Optimal staining reaction for MLH1 of the tonsil using the GA079 RTU system for the Dako Omnis platform based on mAb clone ES05, following the recommended protocol settings using dual linker. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction.

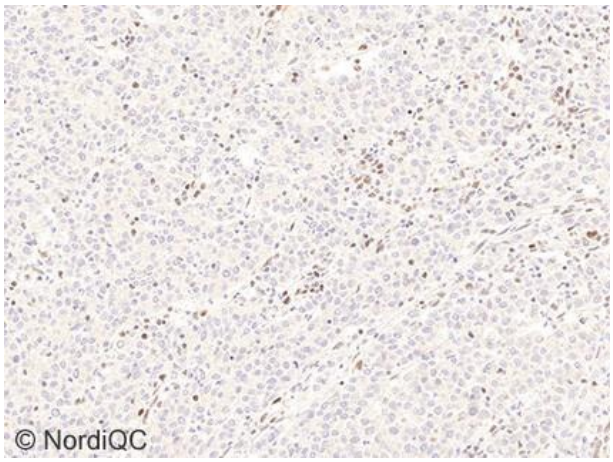


Fig. 5b
Optimal staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 5, with loss of MLH1 using same protocol as in Fig. 5a. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. A faint cytoplasmic reaction not interfering the interpretation is accepted.

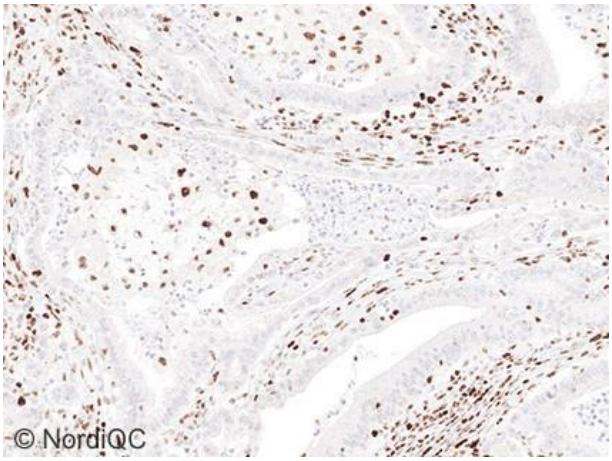


Fig. 6a
Optimal staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 4, with loss of MLH1 using the mAb clone M1 in a RTU format (790-5091) on the Ventana Benchmark Ultra instrument and adding an OptiView Amplification Kit (incubation times 4+4 min.) to the recommended protocol settings (HIER in CC1 for 64 min. and Ab incubation time 24 min.). The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

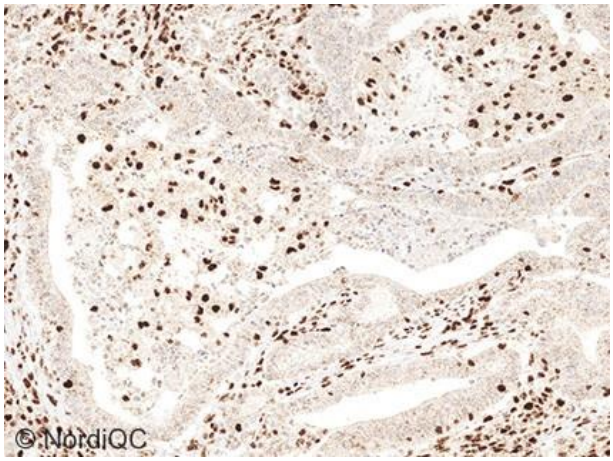


Fig. 6b
Insufficient MLH1 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of MLH1 expression using the mAb clone M1 in a RTU format (790-5091) on the Ventana Benchmark Ultra instrument together with OptiView Visualization system + OptiView Amplification Kit with extended incubation times of 8+8 min. A poor signal-to-noise ratio most likely caused by the too long incubation times of the tyramide based amplification reagents interferes the interpretation by giving a weak cytoplasmic and nuclear staining reaction in the neoplastic cells expected to be negative. Compare with Fig. 6a for optimal result – same area.

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