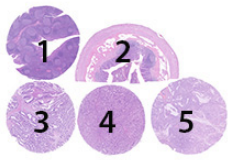
## Assessment Run 56 2019

# Mismatch Repair Protein MLH1 (MLH1)

**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 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. 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 MLH1, run 56 | 270 |
| Number of laboratories returning slides | 259 (96%) |

**Results**
259 laboratories participated in this assessment, 90% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful primary antibodies
- Too low concentration of the primary antibody
- Less sensitive detection systems
- Unexplained technical issues

**Performance history**
This was the fifth NordiQC assessment of MLH1. A significant increase in pass rate was observed (see Table 2).

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

| | Run 13 2005 | Run 30 2010 | Run 40 2014 | Run 49 2017 | Run 56 2019 |
|---|---|---|---|---|---|
| Participants, n= | 25 | 85 | 142 | 224 | 259 |
| Sufficient results | 72% | 57% | 73% | 59% | 90% |

**Conclusion**
The mAb clones **BC23**, **BS29**, **ES05**, **G168-15**, **GM011**, **IHC409** and **M1** could all be used to obtain an optimal staining for MLH1. Irrespective of the clone applied, Heat Induced Epitope Retrieval (HIER) in an alkaline buffer, use of a highly sensitive detection system and careful calibration of the primary antibody were the most important prerequisites for an optimal staining result.
In this assessment and in concordance with previous assessments, the mAb clone **ES05** was very successful, both as concentrate and as Ready-To-Use (RTU) format (Dako/Agilent and Leica/Novocastra). Many laboratories used the RTU **IR079/IS079** (mAb clone **ES05)** intended for the Dako Autostainer on the Dako Omnis, resulting in a drop in both pass rate and proportion of optimal staining results compared to the results on the intended platform. These data show – in concordance with previous findings – that "direct" transfer of original Autostainer protocols to the Dako Omnis must be avoided. Adjustments to key protocol settings as HIER, incubation time in antibody and detection system might be needed and subsequent validation is required. The mAb clone **M1** based RTU systems from Ventana/Roche were all very successful, demonstrating both very high pass rates and proportions of optimal staining results. This was in contrast to the last assessment, where the pass rate was very low due to an unexpected aberrant nuclear staining in the neoplastic cells of one of the colon adenocarcinomas known to lack MLH1

expression. The reason for this aberrant nuclear staining (which has only been reported in rare cases) is at present unknown [1].

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 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 56**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | Suff. OPS[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **BC23** | 1 | Biocare | 1 | 0 | 0 | 0 | - | - |
| mAb clone **BS29** | 1<br>1 | Master Diagnostica<br>Nordic Biosite | 0 | 1 | 1 | 0 | - | - |
| mAb clone **ES05** | 33<br>32 | Novocastra/Leica<br>Dako/Agilent | 40 | 18 | 3 | 4 | 89% | 90% |
| mAb clone **G168-15** | 9<br>4<br>1 | BD Pharmingen<br>Biocare<br>Diagnostic BioSystems | 5 | 5 | 2 | 2 | 71% | 77% |
| mAb clone **G168-728** | 2 | Cell Marque | 0 | 1 | 1 | 0 | - | - |
| mAb clone **GM011** | 2<br>1<br>1 | Nordic Biosite<br>Genemed<br>Sakura Finetek | 2 | 2 | 0 | 0 | - | - |
| mAb clone **IHC409** | 1 | CliniScience | 1 | 0 | 0 | 0 | - | - |
| Ready-To-Use antibodies | | | | | | | | |
| mAb clone **BC23 API 3214** | 1 | Biocare | 1 | 0 | 0 | 0 | - | - |
| mAb clone **BS29 MAD-00726QD** | 2 | Master Diagnostica | 2 | 0 | 0 | 0 | - | - |
| mAb clone **ES05 IR079/IS079** | 35 | Dako/Agilent | 26 | 7 | 1 | 1 | 94% | 100% |
| mAb clone **ES05 IR079/IS079** [3] | 26 | Dako/Agilent | 7 | 13 | 6 | 0 | 77% | - |
| mAb clone **ES05 IR079/IS079** [4] | 7 | Dako/Agilent | 5 | 1 | 1 | 0 | - | - |
| mAb clone **ES05 PA0610** | 4 | Novocastra/Leica | 4 | 0 | 0 | 0 | - | - |
| mAb clone **G168-15 PM220** | 1 | Biocare | 1 | 0 | 0 | 0 | - | - |
| mAb clone **G168-15 BMS033** | 3 | Zytomed | 1 | 1 | 1 | 0 | - | - |
| mAb clone **G168-728 285M-17/285M-18/285M** | 2 | Cell Marque | 0 | 1 | 1 | 0 | - | - |
| mAb clone **M1 760-5091 / 790-5091** | 48 | Ventana/Roche | 40 | 7 | 0 | 1 | 98% | 100% |
| mAb clone **M1 790-4535** [5] | 39 | Ventana/Roche | 27 | 10 | 1 | 1 | 90% | 93% |
| mAb clone **M1 790-4535** [6] | 1 | Ventana/Roche | 1 | 0 | 0 | 0 | - | - |
| mAb clone **MX063 MAB-0838** | 1 | Maixin | 1 | 0 | 0 | 0 | - | - |
| Total | 259 | | 165 | 67 | 18 | 9 | - | |
| Proportion | | | 64% | 26% | 7% | 3% | 90% | |

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on the Dako/Agilent full-automatic platform (Dako Omins)
4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer), but used by laboratories on other platforms.

5) Discontinued product no. Recently substituted by product no. 760-5091 or 790-5091 (US)
6) RTU system developed for the Ventana BenchMark system, but used with the Leica Bond system.

**Detailed analysis of MLH1, Run 56**
The following protocol parameters were central to obtain optimal staining results:

**Concentrated antibodies**
mAb clone **BC23**: One protocol with an optimal result was based on 40 min. HIER using Tris-EDTA / EGTA pH 9 (1/1)*. 45 min. incubation of the primary Ab diluted 1:100 and Zytomed HRP-Polymer (POLHRP-100) as detection system.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **ES05**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1, Ventana) (18/28), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (9/15), Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (4/7), TRS High pH (Dako) (4/7), Tris-EDTA/EGTA pH 9 (1/2), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (1/2) or TRS pH 6.1 (Dako) (1/1) as retrieval buffer. Two laboratories with optimal results did not specify the HIER buffer used. 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, 56 of 62 (90%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **G168-15**: Protocols with optimal results were all based on HIER using CC1 (Ventana) (4/11) or BERS2 (Leica) (1/2), as retrieval buffer. The mAb was typically diluted in the range of 1:20-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 10 of 13 (92%) laboratories produced a sufficient staining result.

mAb clone **GM011**: Protocols with optimal results were all based on HIER using TRS High pH (Dako) (1/3) or Tissue-Tek Genie High pH Antigen Retrieval Solution (Sakura). The mAb was typically diluted in the range of 1:100-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 4 of 4 laboratories produced a sufficient staining result.

mAb clone **IHC409**: One protocol with an optimal result was based on 20 min. HIER using TRS High pH (3-in-1) (Dako), 30 min. incubation of the primary Ab diluted 1:100 and EnVision FLEX+ (Dako, K8002/SM802) as detection system.

Table 3. **Proportion of optimal results for MLH1 for the most commonly used antibodies as concentrate on the four main IHC systems***

| Concentrated antibodies | **Dako Autostainer Link / Classic** | | **Dako OMNIS** | | **Ventana BenchMark GX / XT / Ultra** | | **Leica 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** | 4/5** (80%) | - | 4/7 (57%) | - | 18/28 (64%) | - | 9/15 (60%) | 1/2 |
| mAb clone **G168-15** | - | - | - | - | 4/11 (36%) | - | 1/2 | - |

* 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 **BC23** product no. **API 3214**, Biocare, intelliPATH:
One protocol with an optimal result was based on HIER using Diva Decloaker pH 6.2 in a Pressure Cooker (efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH4 Universal HRP-Polymer (M4U534) as detection system.

mAb clone **ES05**, product no. **MAD-000726QD-7/N**, Master Diagnostica, LabVision:
Protocols with optimal results were based on HIER using EDTA/EGTA pH 9, efficient heating time 20 min. at 95-97°C, 30 min. incubation of the primary Ab and Master Polymer Plus (MAD-000237QK/N) as detection system. Using these protocol settings, 2 of 2 (100%) laboratories produced an optimal staining result.

mAb clone **ES05,** product no. **IR079/IS079,** Dako/Agilent, Autostainer Link / Classic:
Protocols with optimal results were typically based on 10-30 min. HIER using TRS High pH (3-1), 20-30 min. incubation of the primary Ab and a 3-layer EnVision FLEX+ (K8004/DM828) as detection system. Using these protocol settings, 31 of 31 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **ES05,** product no. **PA0610,** Leica/Novocastra, BOND Max / BONDIII:
Protocols with optimal results were typically based on 10-30 min. HIER using BERS2 or 30 min in BERS1, 10-30 min. incubation of the primary Ab and a 3-layer Bond Refine (DS9800) as detection system. Using these protocol settings, 4 of 4 (100%) laboratories produced an optimal staining result.

mAb clone **M1**, product no. **760-5091** or **790-5091**, Ventana/Roche, BenchMark GX / XL / Ultra:
Protocols with optimal results were typically based on 32-64 min. HIER using CC1, 8-60 min. incubation of the primary Ab and a 3-layer OptiView (760-700) or OptiView (760-700) with amplification (760-099 / 860-099) as detection system. Using these protocol settings, 43 of 43 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **M1**, product no. **790-4535**, Ventana/Roche, BenchMark GX / XL / Ultra:
Protocols with optimal results were typically based on 32-92 min. HIER using CC1, 8-44 min. incubation of the primary Ab and a 3-layer OptiView (760-700) or OptiView (760-700) with amplification (760-099 / 860-099) as detection system. Using these protocol settings, 25 of 27 (93%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **MX063**, product no. **MAB-0838**, Maixin, manual:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (Waterbath) for 20 min., 60 min. incubation of the primary Ab and MaxVision III Ultra DAB as detection system.

Table 4 summarises 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 specific IHC stainer device are included.

Table 4. **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 |
| Dako Autostainer Classic/Link mAb ES05 **IR079/IS079** | 100% 15/15 | 93% 14/15 | 90% 18/20 | 60% 12/20 |
| VMS Ultra/XT mAb M1 **760-5091** or **790-5091** | 100% (9/9) | 100% (9/9) | 97% (38/39) | 79% (31/39) |

* 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 >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

**Comments**
In contrast to the previous assessment in 2017 (Run 49), the prevalent feature of an insufficient staining in this assessment was a too weak or completely false negative staining reaction in cells expected to be demonstrated. Too weak or false negative staining reaction was seen in 81% of the insufficient results (22 of 27). The remaining 19% of insufficient results (5 of 27 laboratories) were characterized by false positive staining reaction or staining reaction with poor signal-to-noise ratio (see Fig. 7a and 7b). In run 49 (2017), 67% of the insufficient results (60 of 90 laboratories) were due to false positive nuclear staining in one of the colon carcinomas with known loss of MLH1 or a general poor signal to noise ratio. The aberrant nuclear staining reaction was seen with the mAb clones M1 and G168-728. Similar observations were done with mAb clone M1 in the Canadian Immunohistochemistry Quality Control program (cIQc) in their MLH1 assessment in 2014[1]. No explanation has been given for this reported false positive staining, and more data must be generated to elucidate this aberrant nuclear staining reaction. In the current assessment no false positive nuclear staining reaction was observed with mAb clone M1. 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 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.

34% (89 of 259) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for MLH1. The mAb clone ES05 was the most widely used Ab for demonstration of MLH1 and provided a high proportion of sufficient staining results (see Table 1). Optimal results could be obtained on all four main IHC systems from Dako/Agilent, Leica/Novocastra and Ventana/Roche using the clone as concentrate (see Table 3). In concordance with the previous assessment (Run 49, 2017), the highest proportion of optimal results was seen on the Dako Autostainer with 80%, compared to Ventana Benchmark, Leica Bond and Dako Omnis with 64%, 60% and 57% respectively. On all four platforms, efficient HIER in an alkaline buffer in combination with a sensitive non-biotin-based detection system and a titre in the range of 1:10-1:100 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. For both the Leica Bond and especially the Ventana BenchMark platform the proportions of optimal results have improved significantly from Run 49 to Run 56. On the Ventana BenchMark, the proportion of optimal results rose from 21% to 64% and for the Leica Bond from 33% to 60%. This improvement predominantly relates to the use of both higher concentrations and longer incubation times in mAb clone ES05. On the Ventana BenchMark the mean dilution factor for clone ES05 in Run 49 and Run 56 was 1:44 and 1:34, respectively, for optimal results. Likewise, the mean incubation time rose from 33 min. in Run 49 to 38 min. in Run 56. Similar data were seen on the Leica Bond platform. The mAb clone G168-15 as concentrated format could be used to obtain optimal results on both the Leica and Ventana systems, whereas no laboratories used the concentrated mAb clone G168-15 on the Dako system. On both platforms, optimal protocols were all based on efficient HIER at high pH and a sensitive 3-layer polymer/multimer-based detection system. Antibody titre ranged from 1:20 to 1:50 at the Ventana BenchMark, whereas a dilution factor of 1:200 was used at the Leica Bond. The mAb clone G168-728 used as concentrate was found to be less successful, as a consistent aberrant cytoplasmic staining reaction was seen in nerves and endothelial cells, often together with a too weak specific nuclear staining. The recently introduced mAb clones BC23 (n=1), GM011 (n=4) and IHC409 (n=1) could all produce optimal staining results.

66% (170 of 259) of the laboratories used Abs in RTU formats. The most widely used RTU systems for MLH1 were the Ventana 790-4535 and 760-5091/790-5091 systems based on the mAb clone M1, and the Dako IR079/IS079 system, based on mAb clone ES05 and tailored for the Dako Autostainer. The RTU system 790-4535 has recently been reformulated and launched as the RTU systems 760-5091/790-5091. Despite 790-4535 recently has been discontinued, a significant number of laboratories participated with this system. All three systems provided a high pass rate and proportion of optimal results (see Table 1). Used according to the recommended protocol settings, the 760-5091/790-5091 system had a pass rate of 100% with 100% being optimal (see Table 4). The IR079/IS079 system had a pass rate of 100% with 93% being optimal (see Table 4). Lacking a RTU alternative tailored to the Dako Omnis platform, 26 laboratories used the IR079/IS079 system on the Dako Omnis. Despite using similar protocol settings as recommended for the Dako Autostainer, the pass rate dropped to 77%, with an optimal proportion of only 27%. Similar results were seen in previous assessment (Run 49, 2017) These data suggest that the use of the IR079/IS079 system on the Dako Omnis platform within a laboratory developed (LD) assay, requires profound adjustment of the protocol settings and a "direct" transfer of the original Autostainer protocol cannot be applied (see Fig. 1 – Fig. 5). The mAb clone ES05 based RTU system PA0610 from Leica was assessed for the first time in NordiQC. Four laboratories participated and all provided optimal staining results (see Fig. 6a).

This was the fifth NordiQC assessment of MLH1. A significant increase in pass rate was observed (see Table 2). The pass rate increased from 59% in run 49 (2017) to 90% in run 56 (2019). This was primary due to improved performance of mAb clone M1 (see Fig. 6b). In Run 49 in 2017, the mAb clone M1 based RTU system 790-4535, in contrast to previous assessments, performed poorly. Only 22% of the laboratories obtained sufficient result and no optimal scores. The main reason was an unexpected aberrant nuclear staining reaction of neoplastic cells in one of the colon adenocarcinomas known not to express MLH1. The aberrant nuclear staining was primary seen in certain areas of that tumour. These areas generally accounted for more than 10% of the neoplastic cells and could not be ignored. In previous NordiQC assessments (Run 25, Run 30, and Run 40) the aberrant nuclear staining has not been observed with mAb clone M1, but the Canadian Immunohistochemistry Quality Control program (cIQc) reported in their MLH1 assessment in 2014: "*Four different clones were used. Clone M1 did appear to give false positive staining of core 4, for some labs"* [1]. No explanation was given for this reported false positive staining. Likewise, no explanation could be given for the aberrant nuclear staining seen in NordiQC Run 49 assessment. In the current assessment, using colon carcinomas different from the ones used in Run 49, no aberrant nuclear staining reaction was seen with mAb clone M1 based RTU systems. Both the "old" 790-4535 and the reformulated system 760-5091/790-5091 provided a high pass rate and proportion of optimal results (see Table 1).

**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 the surrounding stromal cells and lymphocytes.

[1] B Gilks, J Garratt and E Torlakovic. Assessors' report for cIQc Run 38: MMR immunostaining (May 2014). Canadian Immunohistochemistry Quality Control Program.

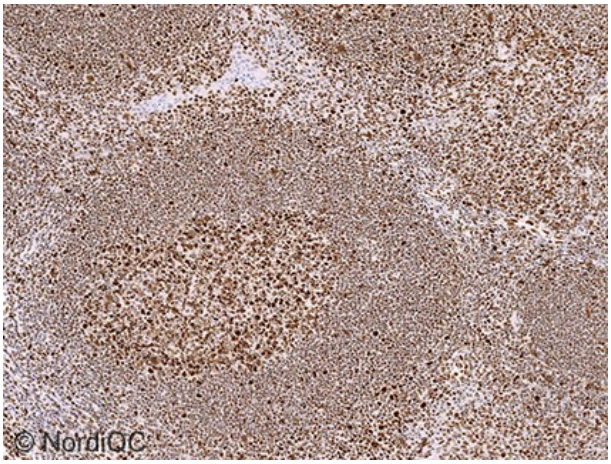



Fig. 1a
Optimal staining reaction for MLH1 of the tonsil using the mAb clone ES05 in a RTU format (IR079/IS079) on the Dako Autostainer instrument using the recommended protocol. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Also compare with Figs. 2a and 3a, same protocol.

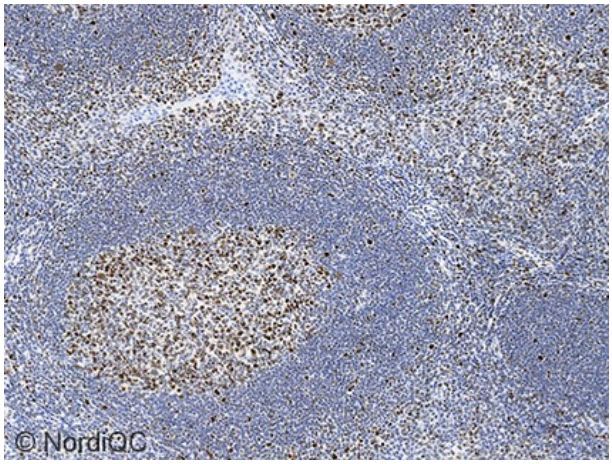



Fig. 1b
Insufficient staining reaction for MLH1 of the tonsil using the mAb clone ES05 in a RTU format (IR079/IS079) as "plug-and-play" on the Dako Omnis. A simple transfer of the Dako Autostainer protocol to the Dako Omnis results in a protocol with too low sensitivity.
Compare with Fig. 1a – same field. 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 and 3b, same protocol.

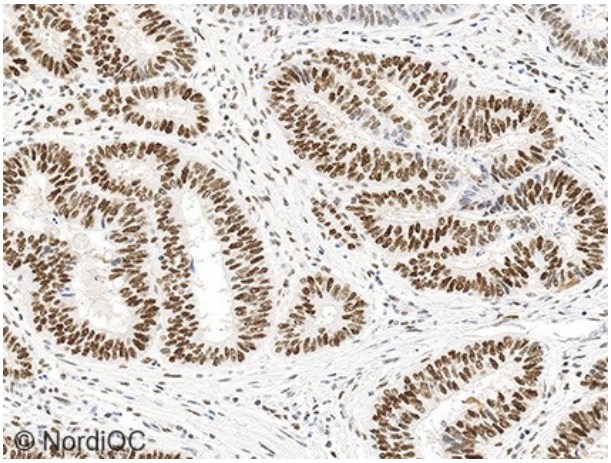



Fig. 2a
Optimal staining reaction for MLH1 of the colon adenocarcinoma tissue core no. 3 with normal MLH1 expression using same protocol as in Fig. 1a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained. No background staining is seen and a distinct nuclear staining reaction in the stromal cells is seen.

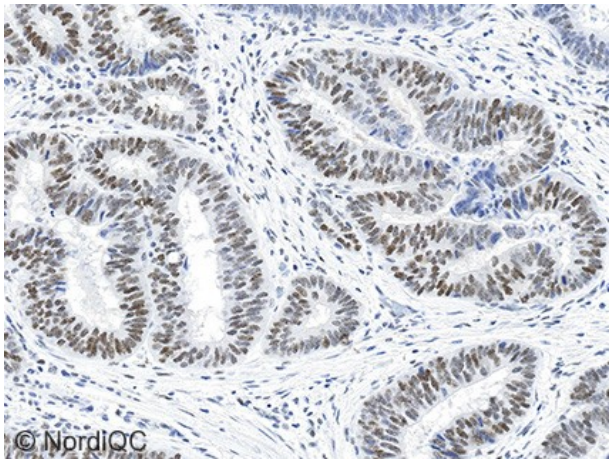



Fig. 2b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma tissue core no. 3 using same protocol as in Fig. 1b – same field as in Fig. 2a. The proportion of positive neoplastic cells and the intensity of the staining reaction are reduced compared to the result in Fig. 2. Virtually no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

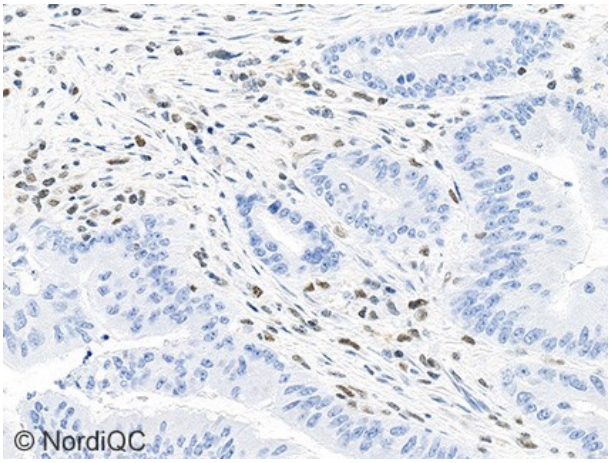



Fig. 3a
Optimal staining reaction for MLH1 of the colon adenocarcinoma no. 5 with loss of MLH1 using same protocol as in Figs. 1a and 2a. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

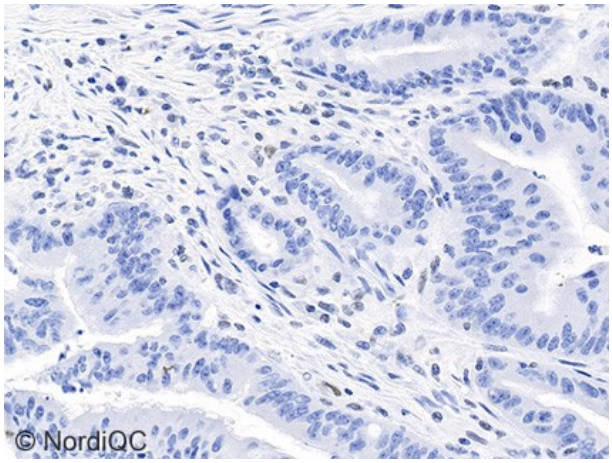



Fig. 3b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma no. 5 with loss of MLH1 using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. No staining reaction in the neoplastic cells is seen, but as virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern cannot reliably be interpreted.

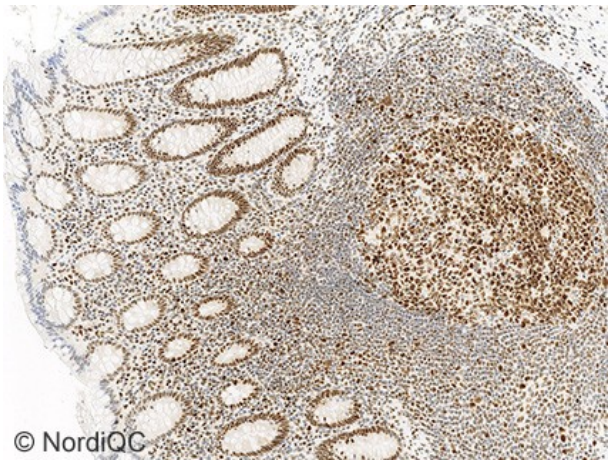



Fig. 4a
Optimal staining reaction for MLH1 of the appendix using the mAb clone ES05 in a RTU format (IR079/IS079) on the Dako Omnis instrument. With careful calibration of the "Autostainer-RTU"-system (IR079/IS079) e.g. prolonged incubation times in HIER and primary Ab, the system can produce optimal result on the Dako Omnis. In mucosa associated lymphatic tissue, 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. Virtually all columnar epithelial cells display a strong nuclear staining reaction.

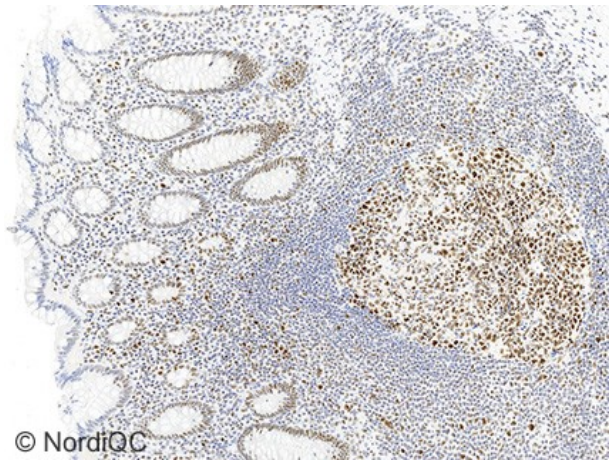



Fig. 4b
Insufficient staining reaction for MLH1 of the appendix using the mAb clone ES05 in a RTU format (IR079/IS079) as "plug-and-play" on the Dako Omnis. A simple transfer of the Dako Autostainer protocol to the Dako Omnis, results in a protocol with too low sensitivity.
Compare with Fig. 4a - same field. Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing a low level of MLH1 are virtually unstained. Similar, columnar epithelial cells display only weak to moderate nuclear staining reaction.

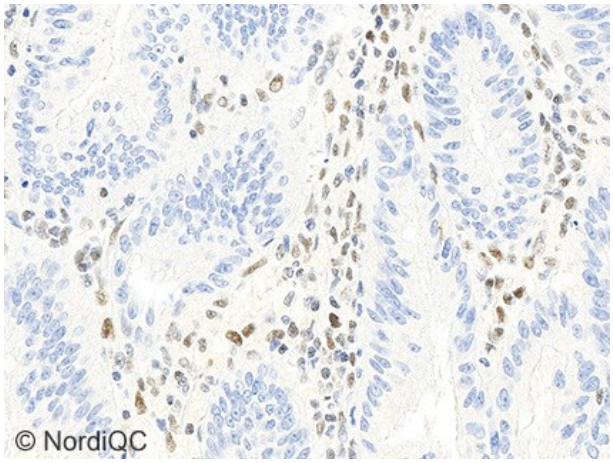



Fig. 5a
Optimal staining reaction for MLH1 of the colon adenocarcinoma no. 5 with loss of MLH1 using same protocol as in Fig. 4a. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

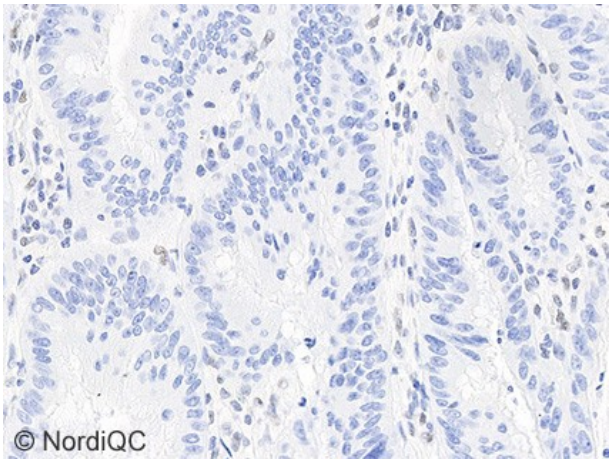



Fig. 5b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma no. 5 with loss of MLH1 using same protocol as in Fig. 4b – same field as in Fig. 5a.
No staining reaction in the neoplastic cells is seen, but as virtually no nuclear staining reaction is seen in the normal stromal cells, the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

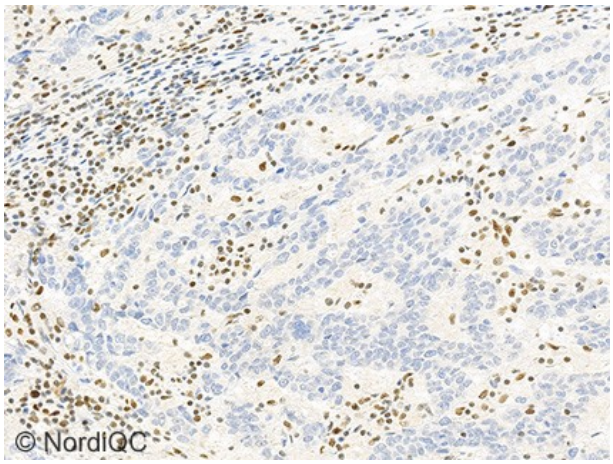



Fig. 6a
Optimal staining reaction for MLH1 of the colon adenocarcinoma no. 4 with loss of MLH1 using the mAb clone ES05 in a RTU format (PA0610) on the Leica Bond instrument using the recommended protocol. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. Compare with Fig. 6b, 7a and 7b – same field.

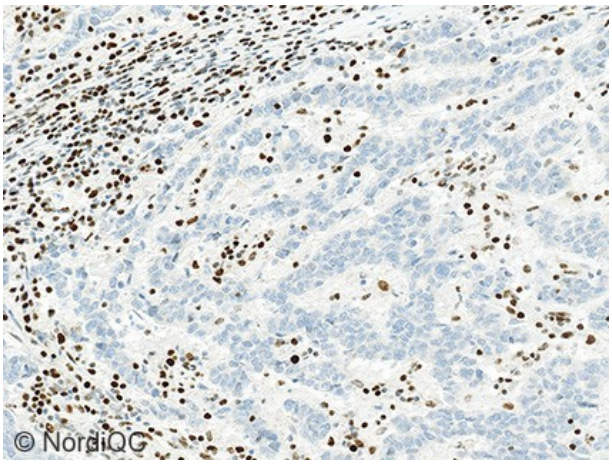



Fig. 6b
Optimal staining reaction for MLH1 of the colon adenocarcinoma no. 4 with loss of MLH1 using the mAb clone M1 in a RTU format (760-5091) on the Ventana BenchMark instrument using the recommended protocol. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. Compare with Fig. 6a, 7a and 7b – same field.

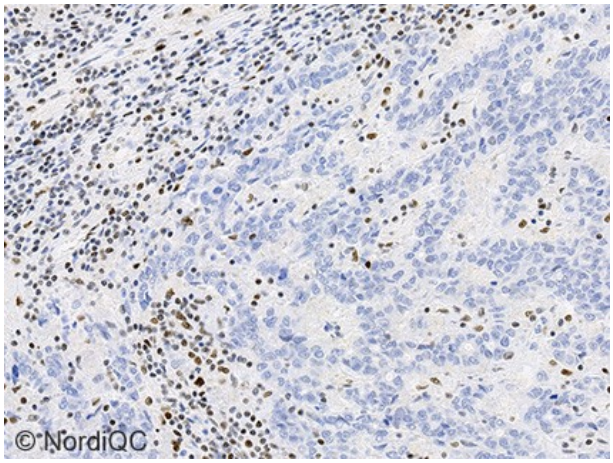



Fig. 7a
Optimal staining reaction for MLH1 of the colon adenocarcinoma no. 4 with loss of MLH1 using the mAb clone ES05 in a RTU format (IR079/IS079) on the Dako Autostainer instrument using the recommended protocol. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. Compare with Fig. 6a, 6b and 7b – same field.

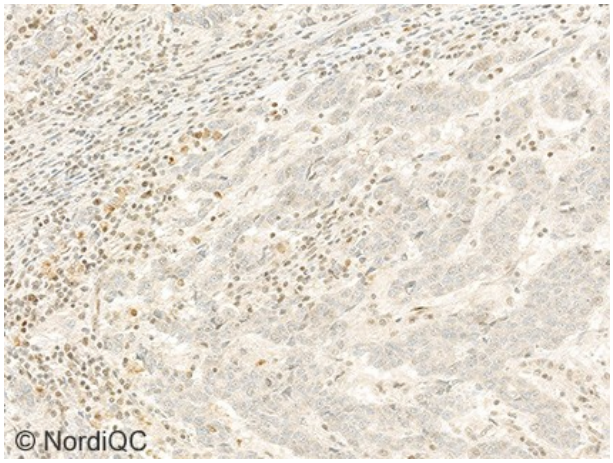



Fig. 7b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma no. 4 with loss of MLH1 using the mAb clone ES05 in a LD assay on the BenchMark in an amplified UltraView protocol. An aberrant cytoplasmic staining reaction in both stromal cells and neoplastic cells complicates the interpretation, as the nuclear staining reaction in the stromal cells cannot be identified with certainty. Compare with Fig. 6a, 6b and 7a – same field.

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