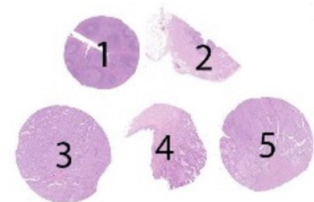
NordiQC

# Assessment Run 76 2026
# 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. Colon adenocarcinoma with loss of MLH1 expression, 5. Endometrial carcinoma with loss of MLH1 expression

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MLH1 staining as optimal included:

- 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, tissue core no. 3*.
- No nuclear staining reaction of neoplastic cells of the colon adenocarcinoma, tissue core no. 4, and endometrial carcinoma, but a distinct nuclear staining reaction in the majority of other cells (stromal cells, lymphocytes etc).
- A weak cytoplasmic staining reaction was accepted.

* Colon adenocarcinoma with normal MLH1 expression showed a slightly heterogenous MLH1 expression and each participant slide was compared to the expression level observed in nearest reference slide.

**KEY POINTS FOR MLH1 IMMONUASSAYS**

- The mAb clones **M1**, **ES05** and **G168-15** were used by 94% of all participants either as a concentrate or RTU, providing a cumulated overall pass rate of 89%, 57% optimal.
- The MLH1 RTU systems **IR/GA079** (mAb clone **ES05**), Dako/Agilent achieved the highest pass rates of 100% when vendor recommended protocol settings (VRPS) were applied.
- The RTU systems **760-5091/790-5091/780-7140** (mAb clone **M1**), Ventana/Roche, and **PA0988** (mAb clone **ES05**), Leica Biosystems, provided superior performance when applied by laboratory modified protocol settings (LMPS) compared to VRPS.
- Data suggests that **790-5091** and **PA0988** might decline during storage and/or handling as older lot numbers had a lower pass and optimal rate, compared to newer lot numbers.
- MLH1 requires a staining protocol with very high sensitivity and preferably HIER in an alkaline buffer.

**Participation**

| Number of laboratories registered for MLH1, run 76 | 454 |
|---|---|
| Number of laboratories returning slides | 373 (82%) |

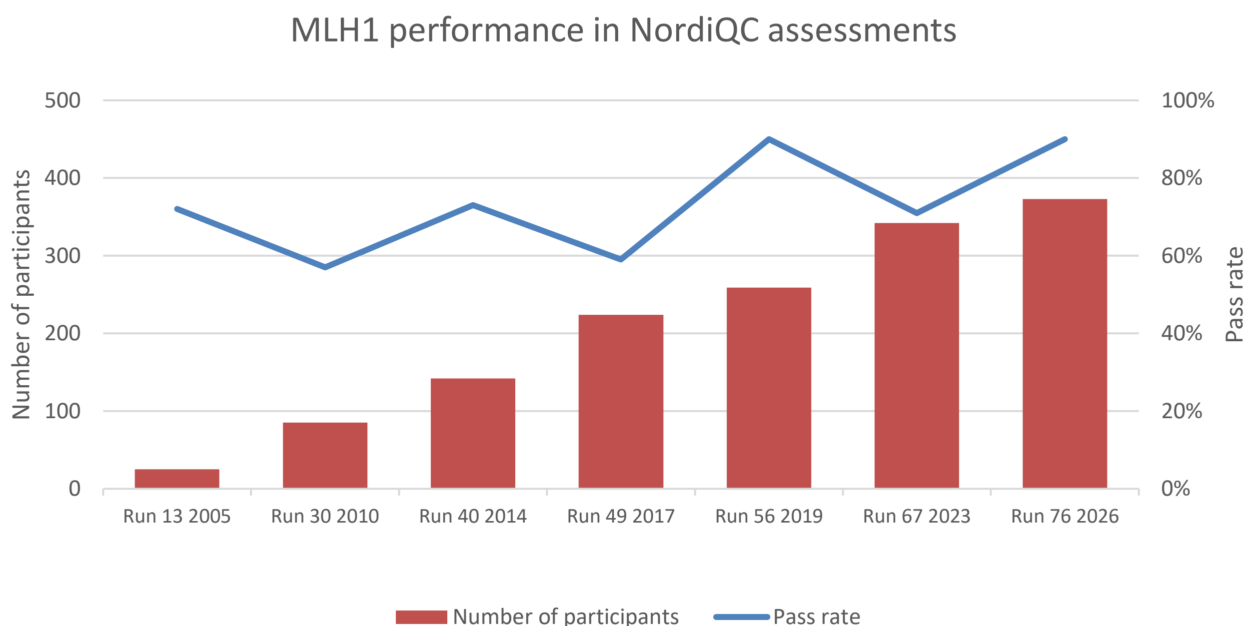
**Results**
At the date of assessment, 82% of the participants had returned the circulated NordiQC slides. In this assessment, run 76, general issues with the Danish postal service affected the distribution and return of slides to/from participants, resulting in a lower number of returned slides compared to previous assessments.
Slides received after the assessment were not included in this report. However, all returned slides were assessed, and participating laboratories with insufficient results received advice.

373 laboratories participated in this assessment. 90% achieved a sufficient mark (optimal or good), see Table 1a. Tables 1b and 1c summarize the antibodies (Abs) used and assessment marks (see page 3).

The most frequent causes of insufficient staining reactions were:
- Use of a less sensitive detection system.
- HIER in citric based buffer.
- Too low concentration of the primary antibody.
- Loss of stability of the primary antibody product.
- Unexplained technical issues.

**Performance history**
This was the seventh NordiQC assessment of MLH1. The pass rate has increased notably compared to the previous run 67 returning to the same level as in run 56 (see Graph 1).

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




**Controls**
Tonsil is 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 the proliferating germinal centre B-cells. Colon adenocarcinoma or endometrial carcinoma with loss of MLH1 expression are 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.

For IHC for Mismatch Repair proteins (MMR) as MLH1 it has to be emphasized that internal positive tissue controls as e.g. normal stromal cells adjacent to the neoplastic cells are preferred to external controls. An observed intact expression of MMR proteins in the internal normal cells together with loss of MMR proteins in the neoplastic cells is of diagnostic importance[1].

[1]Torlakovic, Emina E., et al. "Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee." Applied Immunohistochemistry & Molecular Morphology 23.1 (2015): 1-18.

**Conclusion**
The mAb clones **M1**, **ES05** and **G168-15** could all be used to obtain an optimal staining for MLH1. The vast majority of participants applied an RTU system. Products IR/GA079 (Dako/Agilent) and PA0988 (Leica Biosystems), all based on mAb clone ES05, achieved a very high combined pass rate of 99%, 83% optimal, when used on the intended platform. The lot numbers submitted by participants for PA0988 and 790-5091 (Ventana/Roche) indicate a tendency towards primary antibody products losing sensitivity over time and/or use. To counteract that, the data suggests that PA0988 is performing more robustly when HIER buffer is changed to alkaline BERS2 (91% optimal) instead of vendor recommended low pH BERS1 (44% optimal). Ventana/Roche RTU products 760-5091/790-5091/780-7140 based on mAb clone M1 were more successful when used together with OptiView and OptiView Amplification kit reaching a pass rate of 92% compared to 78% when OptiView alone or UltraView with/without amplification was applied.

Table 1a. **Overall results for MLH1, run 76**

| | n | Optimal | Good | Borderline | Poor | Suff.[1] | OR.[2] |
|---|---|---|---|---|---|---|---|
| Concentrated antibodies | 49 | 27 | 18 | 4 | 0 | 92% | 55% |
| Ready-To-Use antibodies | 324 | 186 | 104 | 33 | 1 | 90% | 57% |
| Total | 373 | 213 | 122 | 37 | 1 | | |
| Proportion | | 57% | 33% | 10% | 0% | 90% | |

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for MLH1, Run 76**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **ES05** | 24<br>14 | Leica Biosystems<br>Dako/Agilent | 20 | 14 | 4 | - | 89% | 53% |
| mAb clone **G168-15** | 3<br>2 | BD Pharmigen<br>Biocare Medical | 3 | 2 | - | - | 100% | 60% |
| mAb clone **13D9-F5** | 1 | Wondfo | - | 1 | - | - | - | - |
| mAb clone **4C9C7** | 1 | Epredia/Thermo Fisher | 1 | - | - | - | - | - |
| mAb clone **BPM6179** | 1 | Biolynx Biotechnology | 1 | - | - | - | - | - |
| mAb clone **BS29** | 1 | Nordic Biosite | 1 | - | - | - | - | - |
| mAb clone **GM011** | 1 | PathnSitu | 1 | - | - | - | - | - |
| mAb clone **ZR347** | 1 | Zeta Corporation | - | 1 | - | - | - | - |
| Conc total | 49 | | 27 | 18 | 4 | 0 | | |
| Proportion | | | 55% | 37% | 8% | 0% | 92% | |

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).

Table 1c. **Ready-To-Use antibodies and assessment marks for MLH1, Run 76**

| Ready-To-Use antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR.[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone **M1 760-5091/ 790-5091/780-7140**[3] | 39 | Ventana/Roche | 5 | 25 | 9 | - | 77% | 13% |
| mAb clone **M1 760-5091/ 790-5091/780-7140**[4] | 104 | Ventana/Roche | 45 | 44 | 15 | - | 86% | 43% |
| mAb clone **ES05 GA079**[3] | 62 | Dako/Agilent | 52 | 10 | - | - | 100% | 84% |
| mAb clone **ES05 GA079**[4] | 27 | Dako/Agilent | 21 | 5 | 1 | - | 96% | 78% |
| mAb clone **ES05 IR079**[3] | 11 | Dako/Agilent | 11 | - | - | - | 100% | 100% |
| mAb clone **ES05 IR079**[4] | 24 | Dako/Agilent | 16 | 5 | 3 | - | 88% | 67% |
| mAb clone **ES05 PA0988**[3] | 9 | Leica Biosystems | 5 | 3 | 1 | - | 89% | 56% |
| mAb clone **ES05 PA0988**[4] | 27 | Leica Biosystems | 21 | 5 | 1 | - | 96% | 78% |
| mAb clone **GM011 8324-C010**[3] | 3 | Sakura Finetek | 2 | 1 | - | - | - | - |
| mAb clone **G168-15 BMS033** | 2 | Zytomed Systems | - | - | 2 | - | - | - |
| mAb clone **G168-15 PM 220 AA** | 1 | Biocare Medical | 1 | - | - | - | - | - |
| mAb clone **G168-15 PDM148** | 1 | Diagnostic BioSystems | - | - | - | 1 | - | - |
| mAb clone **BC23 API 3214 AA** | 2 | Biocare Medical | - | 2 | - | - | - | - |
| mAb clone **QM003 P-M001** | 2 | Quartett | - | 2 | - | - | - | - |
| mAb clone **4F3 B69011** | 1 | Guangzhou Biotron | 1 | - | - | - | - | - |
| mAb clone **BY213 BFM-0576** | 1 | Bioin Biotechnology | 1 | - | - | - | - | - |
| mAb clone **C12A19 CMM-0182** | 1 | Celnovte Biotechnology | 1 | - | - | - | - | - |
| mAb clone **DY49698 4910122** | 1 | Dakewe | - | 1 | - | - | - | - |
| mAb clone **G168-728 BSB 5766** | 1 | BioSB | - | 1 | - | - | - | - |
| mAb clone **GM002 GT2304** | 1 | Gene Tech | 1 | - | - | - | - | - |
| mAb clone **IHC409 IHC409** | 1 | GenomeMe | 1 | - | - | - | - | - |
| mAb clone **MLH1-6467 MAD-000726QDS** | 1 | Master Diagnostica | - | - | 1 | - | - | - |
| mAb clone **MX063 MAB-0838** | 1 | Fuzhou Maixin | 1 | - | - | - | - | - |
| rmAb clone **521I4D5 PA242** | 1 | Abcarta | 1 | - | - | - | - | - |
| RTU total | 324 | | 186 | 104 | 33 | 1 | | |
| Proportion | | | 58% | 32% | 10% | 0% | 90% | |

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 76

The following protocol parameters were central to obtain optimal staining:

### Concentrated antibodies

mAb clone **ES05**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) in an alkaline buffer using either Target Retrieval Solution (TRS) High pH (Dako/Agilent) (1/2), Cell Conditioning 1 (CC1, Ventana/Roche) (10/19) or Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (8/12) 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, 30 of 33 (91%) laboratories produced a sufficient staining (optimal or good), 58% (19/33) optimal.
* (number of optimal results/number of laboratories using this buffer).

mAb clone **G168-15**: Protocols with optimal results were based on HIER using CC1 (Ventana/Roche) (3/5) as retrieval buffer. The mAb was typically diluted in the range of 1:10-1:100 depending on the producer of the antibody (higher concentration needed with BD Pharmigen and lower with Biocare Medical product) and the total sensitivity of the protocol employed. Using these protocol settings, 5 of 5 (100%) laboratories produced a sufficient staining result, 60% (3/5) optimal.

Table 2. **Proportion of optimal results for MLH1 for the most commonly used antibody concentrates on the four main IHC systems by optimal settings as listed above.**

| Concentrated antibody | **Dako/Agilent Autostainer** | | **Dako/Agilent Omnis** | | **Ventana/Roche BenchMark[1]** | | **Leica Biosystems Bond[2]** | |
|---|---|---|---|---|---|---|---|---|
| | TRS pH 9.0 | TRS pH 6.1 | TRS pH 9.0 | TRS pH 6.1 | CC1 pH 8.5 | CC2 pH 6.0 | BERS2 pH 9.0 | BERS1 pH 6.0 |
| mAb clone **ES05** | - | - | 1/2 | - | 10/19 (53%) | - | 8/12 (67%) | - |
| mAb clone **G168-15** | - | - | - | - | 3/5 (60%) | - | - | - |

1) BenchMark GX, Ultra, Ultra plus
2) Bond III, Prime

**Ready-To-Use antibodies and corresponding systems**
mAb clone **M1**, product no. **760-5091/790-5091/780-7140**, Ventana/Roche, BenchMark GX/Ultra/Ultra Plus: Protocols with optimal results were based on HIER using CC1 (efficient heating time 32-92 min. at 95-100°C), 8-44 min. incubation of the primary Ab and OptiView (760-700) with OptiView Amplification (760-099/860-099) as detection system. Using these protocol settings, 52 of 55 (95%) laboratories produced a sufficient staining result, 33 of 55 (60%) scored as optimal.
2 laboratories used the product on a non-intended platform. Data is not included here.

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, 79 of 79 (100%) laboratories produced a sufficient staining result, 67 of 79 (85%) scored as optimal.
7 laboratories used the product on a non-intended platform. Corresponding data is not included here.

mAb clone **ES05**, product no. **IR079**, Dako/Agilent, Autostainer Link 48:
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, 16 of 16 (100%) laboratories produced an optimal staining result.
14 laboratories used the product on a non-intended platform. Data is not included here.

mAb clone **ES05,** product.no. **PA0988**, Leica Biosystems, BOND III/MAX/Prime:
Protocols with optimal results were typically based on HIER in BERS1 (efficient heating time 30 min. at 100-104°C) or BERS2 (efficient heating time 20-30 min. at 95-104°C), 15-30 min. incubation of the primary Ab and BOND Polymer Refine Detection (DS9800/DS9284) as detection system. Using these protocol settings, 32 of 33 (97%) laboratories produced a sufficient staining result, 26 of 33 (79%) scored as optimal.
3 laboratories used the product on a non-intended platform. Data is not included here.

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 |
| Ventana Benchmark mAb clone M1, **760-5091/790-5091/780-7140** | 77% (30/39) | 13% (5/39) | 86% (88/102) | 44% (45/102) |
| Dako Autostainer Link48 mAb clone ES05, **IR079** | 100% (11/11) | 100% (11/11) | 90% (9/10) | 70% (7/10) |
| Dako Omnis mAb clone ES05, **GA079** | 100% (62/62) | 84% (52/62) | 100% (20/20) | 85% (17/20) |
| Leica Bond rmAb clone ES05, **PA0988** | 89% (8/9) | 56% (5/9) | 100% (24/24) | 88% (21/24) |

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation time, 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 a too weak staining reaction in cells expected to be demonstrated, which was seen in 82% (31/38) of the insufficient results. The remaining insufficient results were characterized by granular staining interfering with interpretation, excessive background staining, poor signal-to-noise ratio or false positive staining.
A somewhat peculiar pattern of reduced staining intensity was observed, as the weaker staining reaction was not consistently seen across all tissue cores. This was noticed with both sufficient and insufficient MLH1 staining results. In several cases, reduced sensitivity was confined to one or two cores, most frequently affecting either the tonsil, the colorectal carcinoma with intact MLH1 expression or the endometrial carcinoma with loss of MLH1.
For protocols based on mAb clone M1, there was a tendency for reduced sensitivity to be first observed in core no. 1 (tonsil), core no. 2 (appendix) and/or core no. 3 (colorectal carcinoma with intact MLH1 expression), whereas for mAb clone ES05, reduced sensitivity was in some cases primarily observed in stromal cells in core no. 5 (endometrial carcinoma with loss of MLH1), while other tissue components showed near expected staining intensity. This might illustrate how different clones can be susceptible to different variations in tissue handling process.

In general, most laboratories were able to demonstrate MLH1 in cells with high-level antigen expression, such as proliferating germinal centre B-cells in the tonsil and basal epithelial cells in the colorectal mucosa. In contrast, demonstration of MLH1 in cells with low-level antigen expression, including resting mantle zone B-cells and stromal cells intermingled with tumor 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.

13% (49/373) of the laboratories used Abs in concentrated format within laboratory developed (LD) assays for MLH1, of which 92% (44/48) achieved a sufficient result and 554% (27/49) were assessed as optimal. Optimal staining results could be obtained with the mAb clones ES05, G168-15, BS29, GM011, BPM6179 and 4C9C7 (see Table 1b).

The mAb clone **ES05** was the most widely used concentrated antibody (78%, 38/49) and provided a high proportion of sufficient results (89%, 34/38), with 53% (20/38) assessed as optimal. Optimal results could be obtained on all main fully automated staining platforms from Dako/Agilent, Ventana/Roche and Leica Biosystems. All optimal protocols were based on HIER in an alkaline buffer combined with highly sensitive 3- or 4-step detection systems, including FLEX+, Bond Refine and OptiView with OptiView Amplification Kit. A tendency towards slightly lower dilution factors (df) for sufficient results was observed for the Dako/Agilent product M3640 (mean df 1:33, n=12) compared to the Leica Biosystems product NCL-L-MLH1 (mean df 1:51, n=22).

The mAb clone **G168-15** as a concentrate was, as in previous assessments, only used on the Ventana BenchMark platform and provided sufficient results in all cases (5/5). Although based on limited data, the antibody produced by BD Pharmingen (550838) appeared to possibly require a lower dilution factor for optimal results (1:10-1:50) compared to the product from Biocare Medical (CM 220 AK, BK, CK, 1:50-1:100). All optimal protocols were based on HIER in high pH CC1 buffer for 48-64 min, antibody incubation for 32-64 min and the use of OptiView with OptiView Amplification Kit as a detection system.

As RTU systems for MLH1 are available for all main IHC platforms, the majority of participants (87%, 324/373) used RTU assays.

The Ventana/Roche RTU systems **760-5091/790-5091/780-7140** based on mAb clone **M1** were the most widely used, applied by 38% (143/373) of participants, and provided a combined pass rate of 83% (119/143), with 35% (50/143) assessed as optimal. When applied according to vendor recommended protocol settings (VRPS) using OptiView as detection system, the pass rate was 77% (30/39), with 13% (5/39) being scored as optimal. Data suggests the stability of the primary antibody might have an impact by prolonged storage and/or handling. Among participants applying VRPS, all optimal results were achieved using relatively newer antibody lot numbers (all lot numbers reported – M03050-N16488 – optimal results starting from lot number N06106). Proportion of sufficient results in the same participant cohort within lot numbers starting with "M" was significantly lower (54%, 7/13) compared to participants reporting the use of a lot number starting with "N" (88%, 22/25). It has to be noted that laboratories are asked to submit their protocol data up to 2 months before receiving the slides for staining and not all update necessary information, including used lot number, when these have changed by the time of staining the NordiQC slide. Nevertheless, the results of this assessment illustrate the technical difficulties laboratories face with less robust assay performance as participants using exactly the same protocol settings display significantly different results (see Figs. 1b-4b, lot number M08499, compared to 5a-5b, lot

number N10029). The majority of participants modified the protocol including changes in HIER time, primary Ab incubation time and detection system which overall slightly improved the pass rate to 86% (87/101), 44% (44/101) optimal. As decline in stability of the primary antibody is expressed as loss of sensitivity, the most successful modification was simply adding an OptiView Amplification Kit to the VRPS (HIER in CC1 for 64 min. and antibody incubation for 24 min.), which raised the pass rate to 96% (23/24), 67% (16/24) optimal (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 which can interfere with interpretation, known to be one of the caveats of using the tyramide based amplification system. In this assessment, 74% (20/27) of downgraded results (good or borderline) for participants applying the OptiView Amplification Kit with mAb clone M1 were partly and 56% (15/27) solely attributed to granular staining interfering with interpretation (see Fig. 6b).

The Dako/Agilent RTU system **GA079** based on mAb clone **ES05** for the Dako Omnis platform was used by 24% (89/373) of participants and provided a very high pass rate of 99% (88/89), with 82% (73/89) assessed as optimal (see Figs. 1a-4a). The single insufficient result was obtained using the product on a non-intended platform. This means all slides stained on the Dako Omnis platform with GA079 were assessed as sufficient. VRPS and LMPS achieved almost identical high proportions of optimal results (84-85%, see Table 3). Main modifications to protocols were change of incubation time for antibody and/or linker(s) and changing from the recommended Flex++ detection system with dual linker to using Flex+ with only Mouse linker.

Dako/Agilent product **IR079** based on mAb clone **ES05** and intended for use on the Dako Autostainer staining platform reached a significantly higher overall pass rate of 91% (32/35) compared to results in the previous run 67 where the pass rate was 55% (27/49). This improvement was largely attributable to the majority of participants previously using the IR079 product on the Dako Omnis changing their antibody product to GA079. All laboratories applying the system according to VRPS achieved optimal results, while LMPS also provided a high pass rate of 90% (9/10), with the only slide with a borderline mark being subjected to HIER in low ph. As previously, IR079 was still the most deviantly used RTU product, with 14 participants applying it on a fully automated stainer platform. Although the pass and optimal rate for IR079 on other platforms in this run was relatively high, being 86% (12/14) and 64% (9/14), respectively, 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. Modifications can be very successful but may also generate sub-optimal or aberrant results and therefore must be carefully monitored.

The Leica Biosystems RTU system **PA0988** based on mAb clone **ES05** provided a high overall pass rate of 94% (34/36), with 72% (26/36) optimal. Although the dataset was smaller, a similar tendency of loss of stability of primary antibody was seen for PA0988 as for 790-5091 from Ventana/Roche. Participants applying VRPS reported using lot numbers in the range of 83106-86752, with all 4 participants in the 85490-86752 range achieving an optimal mark (see Fig. 7a). For the former lot numbers, 83106-84823, the optimal rate was 20% (1/5) and 1 participant assessed as borderline (see Fig. 7b). For PA0988, the loss in sensitivity was not as substantial as vast majority still produced a sufficient result, however weaker than expected. Changing the HIER buffer from vendor recommended low pH BERS1 to high pH BERS2 seemed to have compensated the loss in staining intensity thus overcoming possible impact on staining quality from primary antibody storage and handling. Irrespective of other protocol parameters, the proportion of sufficient and optimal results amongst participants applying HIER in high pH buffer BERS2 were 100% (22/22) and 91% (20/22), respectively, compared to 89% (8/9) and 44% (4/9) when using BERS1.

**Summary**

This was the seventh NordiQC assessment of MLH1 and it provided an overall pass rate of 90%, which is markedly higher than the 70% observed in the previous assessment run 67 in 2023. The vast majority of participants (87%, 324/373) used an RTU assay. The high proportion of laboratories applying RTU systems tailored to their respective platforms likely contributed to the improved overall performance. Although in some cases a peculiar pattern of higher loss of sensitivity in single cores was seen, in general it is likely a slightly more robust tissue micro-array was circulated compared to the previous run. 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.

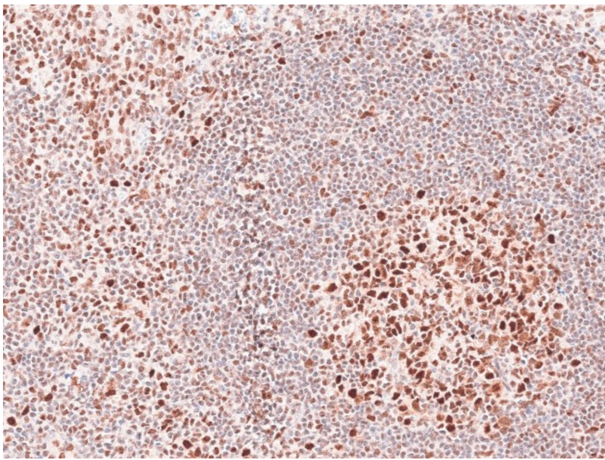


Fig. 1a
Optimal staining reaction for MLH1 of the tonsil using the the Dako/Agilent RTU system GA079 based on mAb clone ES05 on the Dako Omnis staining platform according to vendor recommended protocol settings based on HIER in TRS High pH and FLEX++ detection system with dual linker - same protocol used in Figs. 2a-4a. 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.

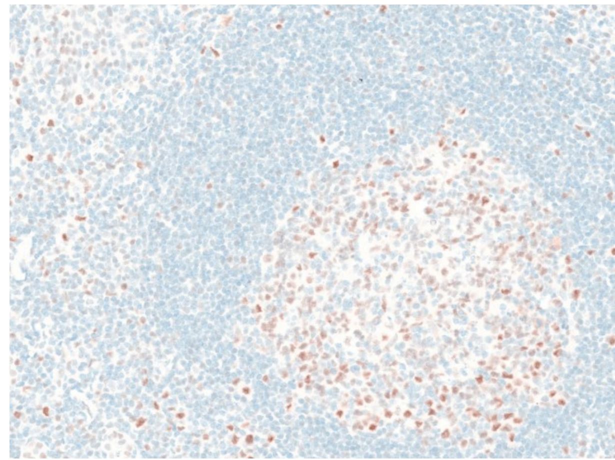


Fig. 1b
Insufficient staining reaction for MLH1 of the tonsil using the Ventana/Roche RTU system 790-5091 based on mAb clone M1, lot number M08499, on the Ventana Benchmark Ultra instrument following vendor recommended protocol settings - same protocol used in Figs. 2b-4b. Compare with Fig. 1a – same 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 Fig. 5a – same protocol with sufficient result.

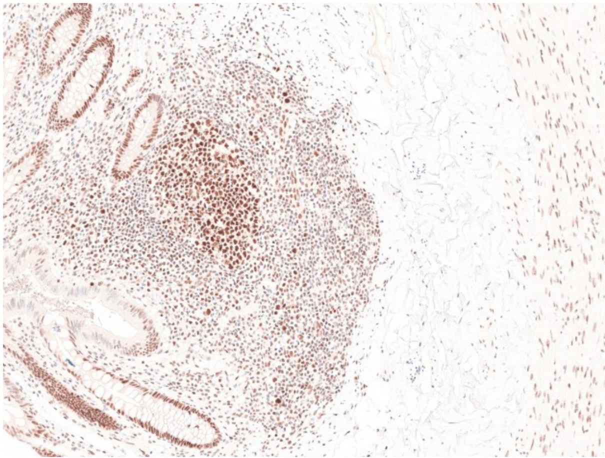


Fig. 2a
Optimal staining reaction for MLH1 of the appendix using the same protocol as in Fig. 1a. Virtually all smooth muscle cells and mantle zone B-cells in the mucosa-associated lymphoid tissue show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells and virtually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

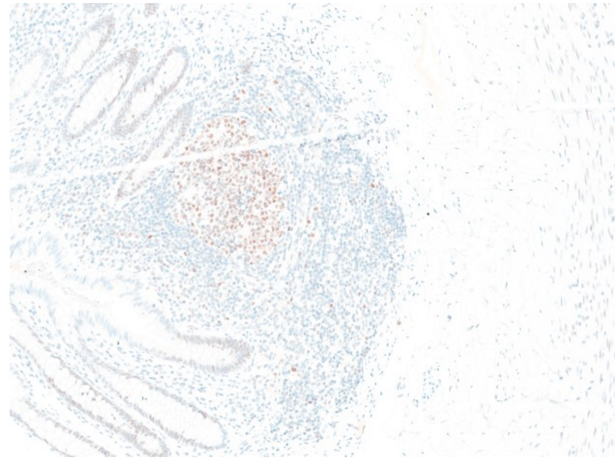


Fig. 2b
Insufficient staining reaction for MLH1 of the appendix using the same protocol as in Fig. 1b. Only the basal parts of the crypts and the germinal centre B-cells are demonstrated, while the mantle zone B-cells and smooth muscle cells expressing a low level of MLH1 are virtually unstained. Similar, columnar epithelial cells display only weak to moderate nuclear staining reaction.

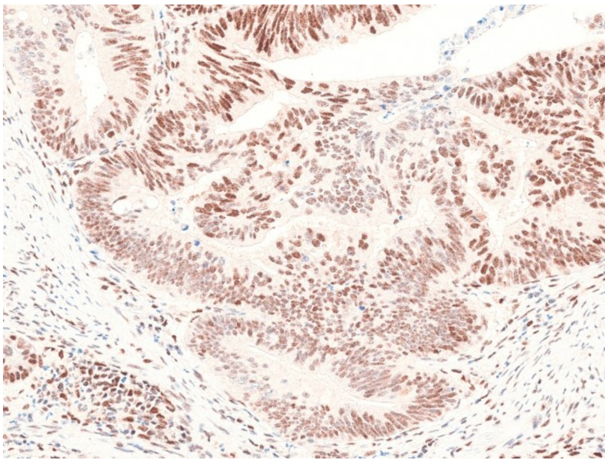


Fig. 3a
Optimal staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 3, with normal MLH1 expression using same protocol as in Figs. 1a-2a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. Stromal cells show a distinct nuclear staining reaction serving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

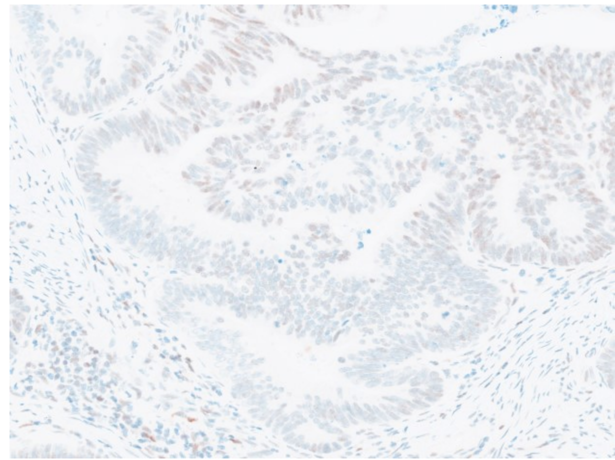


Fig. 3b
Insufficient staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 3, using same protocol as in Figs. 1b-2b. The proportion of positive neoplastic cells and the intensity of the staining reaction are significantly reduced compared to the result in Fig. 3a. Only a weak staining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

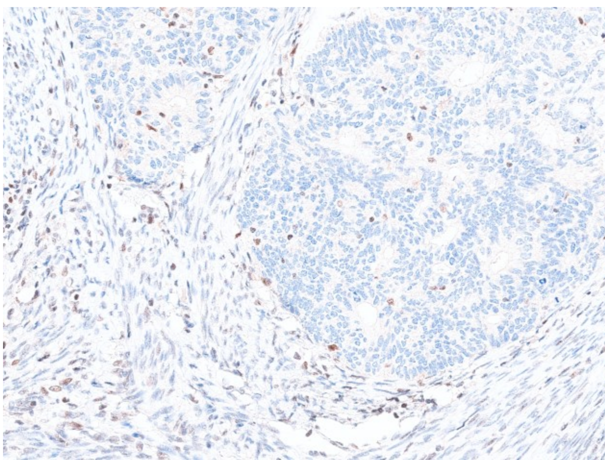


Fig. 4a
Optimal staining reaction for MLH1 of the endometrial carcinoma with loss of MLH1 using same protocol as in Figs. 1a-3a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

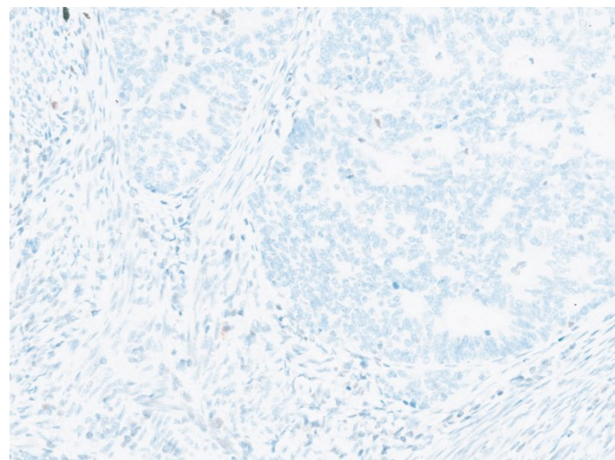


Fig. 4b
Insufficient staining reaction for MLH1 of the endometrial carcinoma with loss of MLH1 using same protocol as in Figs. 1b-3b. No staining reaction in the neoplastic cells is seen, but as virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern cannot reliably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

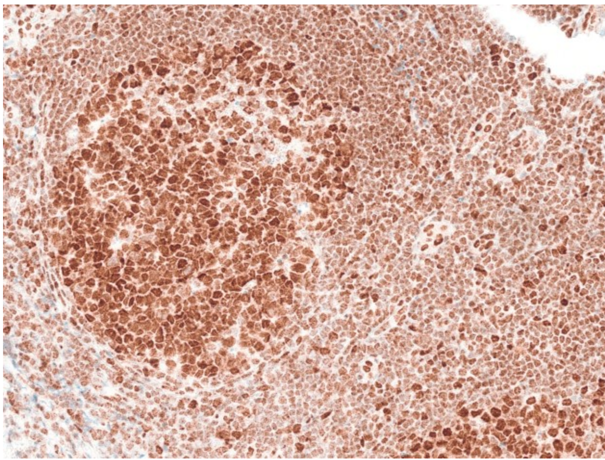


Fig. 5a
Sufficient staining reaction for MLH1 of the tonsil using the Ventana/Roche RTU system 790-5091 based on mAb clone M1, lot number N10029, on the Ventana Benchmark Ultra instrument according to vendor recommended protocol settings - same protocol used in Fig. 5b. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Compare to Fig. 1b – same core and protocol settings, older lot number.

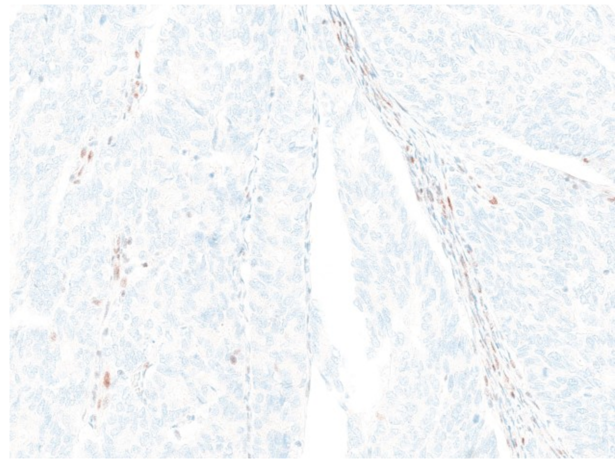


Fig. 5b
Sufficient staining reaction for MLH1 of the endometrial carcinoma, with loss of MLH1 – same assay as in Fig. 5a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. Compare to Fig. 4b – same core and protocol settings, older lot number.

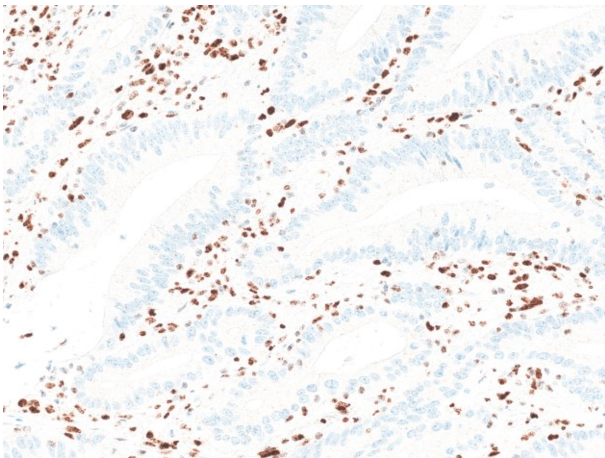


Fig. 6a
Optimal staining reaction for MLH1 of the colon adenocarcinoma, tissue core no. 4, with loss of MLH1 using the mAb clone M1 in a RTU format (790-5091) on the Ventana Benchmark Ultra instrument and adding an OptiView Amplification Kit (incubation times 4+4 min.) to the recommended protocol settings (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.

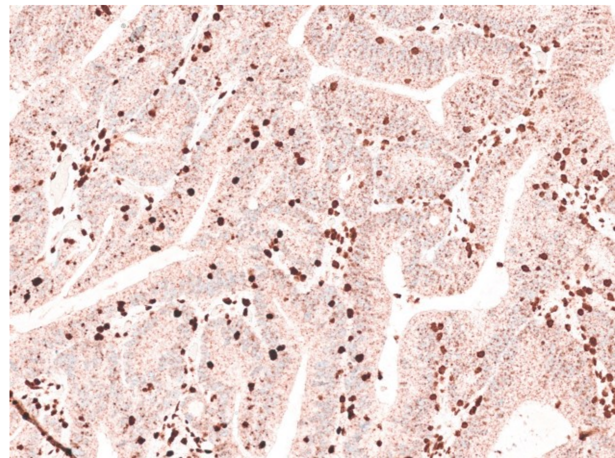


Fig. 6b
Insufficient MLH1 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of MLH1 expression using the mAb clone M1 in a RTU format (790-5091) on the Ventana Benchmark Ultra instrument applying OptiView with OptiView Amplification Kit as detection system. A granular staining reaction most likely caused by the tyramide based amplification reagents interferes the interpretation by partly covering the neoplastic cells expected to be negative. Compare with Fig. 6a for optimal result – same area.

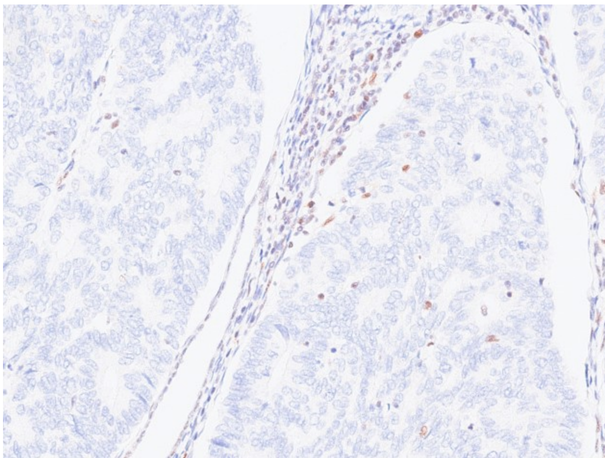


Fig. 7a
Optimal staining reaction for MLH1 of the endometrial carcinoma, tissue core no. 5, with loss of MLH1 using the mAb clone ES05 in a RTU format (PA0988), lot number 85490, on the Leica BOND Prime instrument according to the vendor recommended protocol settings. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

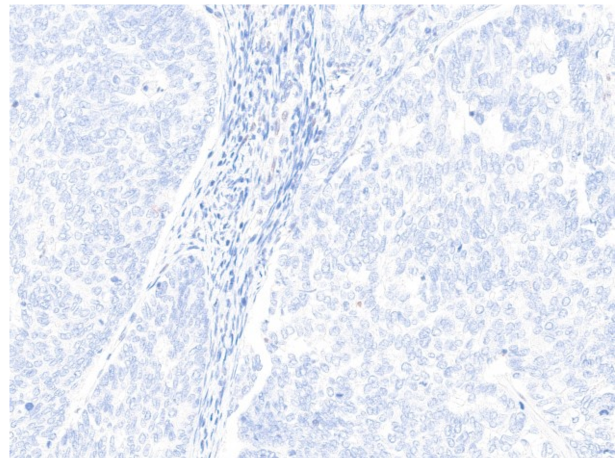


Fig. 7b
Insufficient MLH1 staining reaction of the endometrial carcinoma, tissue core no. 5, with loss of MLH1 using the mAb clone ES05 in a RTU format (PA0988), lot number 84823, on the Leica BOND Prime instrument according to the vendor recommended protocol settings. No staining reaction in the neoplastic cells is seen and only very faint or virtually no nuclear staining reaction is seen in the normal stromal cells or intermingling lymphocytes. Compare to Fig. 7a – same core and protocol settings, newer lot number.

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