Assessment Run 68 2023

Mismatch Repair Protein MSH2 (MSH2)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for MSH2 status in colon adenocarcinomas. Mutation of the MSH2 gene gives rise to a dysfunctional mismatch repair system and is characterized by absence of the MSH2 protein with loss of nuclear expression in neoplastic cells.

Material
The slide to be stained for MSH2 comprised:

1. Appendix, 2. Tonsil, 3. Colon adenocarcinoma with normal MSH2 expression, 4-5. Colon adenocarcinoma with loss of MSH2 expression.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MSH2 staining as optimal were:

- An at least weak to moderate distinct nuclear staining reaction of virtually all smooth muscle and stromal 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, distinct nuclear staining reaction of the germinal centre B-cells in the tonsil.
- A moderate to strong, distinct nuclear staining reaction of all neoplastic cells in the microsatellite stable (MSS) colon adenocarcinoma no. 3.
- No nuclear staining reaction of the neoplastic cells in the MSH2 mismatch repair deficient (MMRd) colon adenocarcinomas no. 4 and no. 5. Importantly, the internal tissue control e.g., all stromal and lymphatic cells in these two cores showing an at least moderate and distinct nuclear staining reaction.

A weak cytoplasmic staining reaction was accepted providing that it did not compromise the interpretation.

Participation

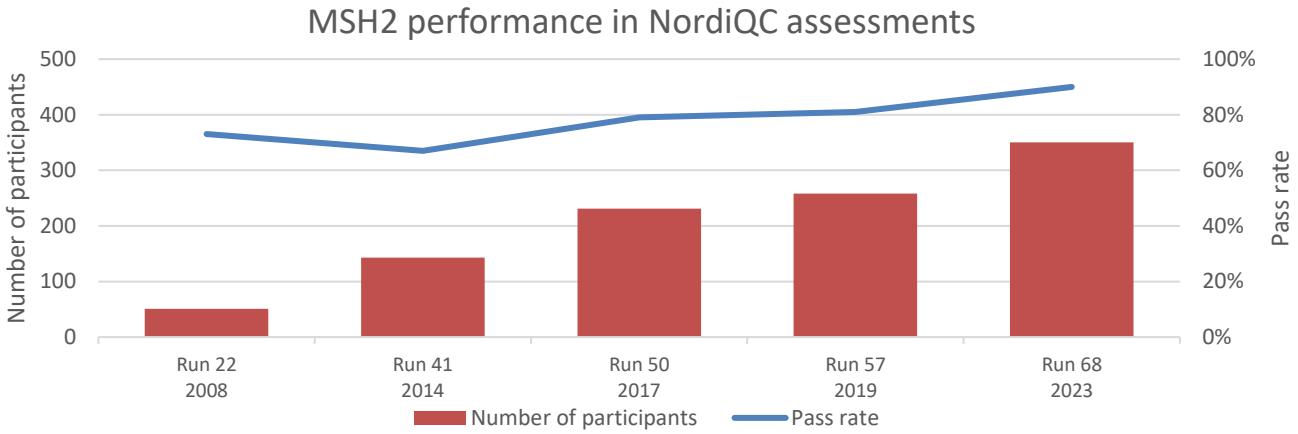
Number of laboratories registered for MSH2, run 68	380
Number of laboratories returning slides	350 (92%)

Results
350 laboratories participated in this assessment and 316 (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 reaction were:
- Insufficient Heat Induced Epitope Retrieval (HIER)
- Use of less sensitive detection systems
- Too diluted primary antibody

Performance history
This was the fifth NordiQC assessment of MSH2. The pass rate has improved over the years and in this run 68 (2023), significantly compared to the previous assessment run 57 (2019), 90% and 81%, respectively (see Graph 1).

Graph 1. Proportion of sufficient results for MSH2 in the five NordiQC runs performed



Conclusion
The most used primary antibodies based on the mAb clones **FE11**, **G219-1129 and 79H11** are all recommendable for demonstration of MSH2. Other antibody clones could also be used to obtain an optimal staining result for MSH2 (see Table 1, page 3). Irrespective of the clone applied, HIER in an alkaline buffer and use of a sensitive and specific polymer/multimer based detection system gave the highest proportion of optimal results.
In this assessment, a significant number of laboratories (88%) used a Ready-To-Use (RTU) system/format for detection of MSH2. The RTU systems GA085 based mAb clone FE11 (Omnis, Dako/Agilent), PA0989 based on the mAb clone 79H11 (Bond III/MAX, Leica Biosystems) and 760-5093 based on the mAb clone G219-1129 (Benchmark Ultra, Ventana/Roche) provided superior results using vendor recommended protocol settings on the fully automated platforms compared to the performance for protocols based on concentrated Abs. Grouped together, all protocols (81/81) were assessed as sufficient and for the RTU system PA0989 (Leica Biosystems) a remarkable proportion of 92% (12/13) of optimal results was obtained. Using vendor recommended protocol settings, the RTU systems IR084 based mAb clone FE11 (Autostainer, Dako/Agilent) also provided a high proportion of sufficient and optimal results, 94% and 69%, respectively. Due to the robustness and the general high quality performance of these RTU systems (using all protocol settings), the overall pass rate increased in this run 68 to 90% (see Graph 1). The pass rate for RTU systems/formats and Laboratory developed assays (LD) was 93% and 73%, respectively.
Tonsil is recommendable as external positive tissue control for MSH2 and to monitor the IHC test reproducibility with focus on the level of analytical sensitivity. However, for IHC for Mismatch Repair proteins (MMR) as MSH2 it is essential that internal positive tissue controls, e.g. normal stromal cells adjacent to the neoplastic cells, are preferred to external controls - 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]

Table 1. **Antibodies and assessment marks for MSH2, run 68**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **FE11**	4 2 2 1 1	Dako/Agilent BioCare Medical Calbiochem Biozol Zytomed Systems	4	3	3	0	70%	40%
mAb clone **G219-1129**	16 4 1 1	Cell Marque BD Biosciences Monosan Immunologic	8	6	7	1	64%	37%
mAb clone **79H11**	3	Leica Biosystems	2	1	0	0	-	-
mAb clone **BPM6143**	2	Biolynx Technologies	1	1	0	0	-	-
rmAb clone **RED2**	1 1	Epitomics Bejing Zhongshang	2	0	0	0	-	-
rmAb clone **QR010**	1	Quartett	1	0	0	0	-	-
rmAb clone **ZR260**	1	Zeta Corporation	1	0	0	0	-	-
Conc total	41		19	11	10	1	73%	46%
Ready-To-Use antibodies								
mAb clone **25D12 PA0048#**	4	Leica Biosystems	3	0	0	1	-	-
mAb clone **79H11 PA0989[3]**	13	Leica Biosystems	12	1	0	0	100%	92%
mAb clone **79H11 PA0989[4]**	10	Leica Biosystems	6	3	1	0	90%	60%
mAb clone **FE11 IR085[3]**	16	Dako/Agilent	11	4	1	0	94%	69%
mAb clone **FE11 IR085[4]**	34	Dako/Agilent	20	9	4	1	85%	59%
mAb clone **FE11 GA085[3]**	35	Dako/Agilent	16	19	0	0	100%	46%
mAb clone **FE11 GA085[4]**	16	Dako/Agilent	8	8	0	0	100%	50%
mAb clone **FE11 MSG031**	2	Zytomed Systems	0	1	1	0	-	-
mAb clone **FE11 MAD-00677QD**	2	Master Diagnostica	0	0	2	0	-	-
mAb clone **FE11 PM219**	1	Biocare Medical	0	1	0	0	-	-
mAb clone **MX061 MAB-0836**	2	Fuzhou Maixin	1	1	0	0	-	-
mAb clone **C2G3 CMM-0191**	1	Celnovte tech.	1	0	0	0	-	-
mAb clone **G219-1129 760-5093[3]**	33	Ventana/Roche	26	7	0	0	100%	79%
mAb clone **G219-1129 760-5093[4]**	119	Ventana/Roche	84	27	8	0	93%	71%
mAb clone **G219-1129 286M-17/18**	14	Cell Marque	6	6	1	1	86%	43%
rmAb clone **SP46 AN743**	1	BioGenex	0	0	0	1	-	-
rmAb clone **RED2 8327-C010**	1	Sakura Finetek	0	1	0	0	-	-
rmAb clone **RED2 01.09.70.13.01.01**	1	Zybio	1	0	0	0	-	-
rmAb clone **RED2 BFM-0133**	1	Bioin Biotechnology	0	1	0	0	-	-
mAb clone **644G5A4 PA194**	1	Abcarta	1	0	0	0	-	-

mAb clone **DGM060 DGM060**	1	Shanghai DG Diagnology Tec	0	0	1	0	-	-
mAb clone **H3Y7 DTBL0216901**	1	DaTe Bioengineering Technology	1	0	0	0	-	-
RTU total	309		197	89	19	4	93%	64%
Total	350		216	100	29	5	-	
Proportion			62%	29%	8%	1%	91%	

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).
#Product discontinued.

Detailed analysis of MSH2, Run 68
The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies
mAb clone **FE11**: Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (3/5)* or Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (1/1) as retrieval buffer. The mAb was diluted in the range of 1:10-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings, 6 of 6 (100%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **G219-1129**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1; Ventana/Roche) (7/13) or BERS2 (Leica Biosystems) (1/5) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 8 of 9 (89%) laboratories produced a sufficient staining result.

mAb clone **79H11**: Protocols with optimal results were based on HIER using BERS2 (Leica Biosystems) (2/3) as retrieval buffer. The mAb was diluted in the range of 1:40-1:300 and Bond Refine (Leica Biosystems) was used as the detection system.

mAb clone **BPM6143**: One protocol with an optimal result was based on HIER using Antigen Retrieval 2 (1/2) (Biolynx Technologies) as retrieval buffer. The mAb was diluted 1:10 and BXV Visalization System (Biolynx Technologies) was used as the detection system.

rmAb clone **RED2**: Two protocols with optimal results were based on HIER using CC1 (Ventana/Roche) (1/1) or Tris-EDTA pH 9 (1/1) as retrieval buffer and OptiView (Ventana/Roche) or GTVision (Gene Tech), respectively, were used as the detection system. The mAb was diluted in the range of 1:100-1:300.

rmAb clone **QR010**: One protocol with an optimal result was based on HIER using Bond Epitope Retrieval Solution 1 (BERS1; Leica Biosystems) as retrieval buffer. The mAb was diluted 1:200 and Bond Refined (Leica Biosystems) was used as the detection system.

rmAb clone **ZR260**: One protocol with an optimal result was based on HIER using BERS2 (Leica Biosystems) as retrieval buffer. The mAb was diluted 1:100 and Zeta Universal HRP Polymer Detection (Zeta Corporation) was used as the detection system.

Table 2. **Proportion of optimal results for MSH2 for the two most commonly used antibody concentrates on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark Ultra**		**Leica Biosystems Bond III**	
	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 **FE11**	-**	-	1/1	-	0/1	-	3/5 (60%)	-
mAb clone **G219-1129**	-	-	-	-	5/6 (83%)	-	1/2	0/1

* 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 **FE11**, product no. **IR085**, Dako/Agilent, Autostainer:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time for 20 min. at 95-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8002/K8021) as detection system. Using these protocol settings, 19 of 20 (95%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **FE11**, product no. **GA085**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time for 30 min. at 97°C), 20 min. incubation of the primary Ab and EnVision FLEX (GV800) with Dual Linkers (GV821/ GV809) as detection system. Using these protocol settings, 34 of 34 (100%) laboratories produced a sufficient staining result.

mAb clone **G219-1129,** product no. **760-5093**, Ventana/Roche, BenchMark Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 12-32 min. incubation of the primary Ab and OptiView (760-700) +/- amplification kit (760-099 / 860-099) as detection systems. Using these protocol settings, 112 of 115 (97%) laboratories produced a sufficient staining result.

mAb clone **79H11,** product no. **PA0989,** Leica Biosystem, Bond III/MAX:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20 min. at 95-100°C), 15 min. incubation of the primary Ab and Bond Refine (DS9800) as detection system. Using these protocol settings, 13 of 13 (100%) laboratories produced a sufficient staining result.

mAb clone **MX061,** product no. **MAB-0836,** Fuzhou Maixin, Titan S:
One protocol with an optimal result was based on HIER using DNS buffer (efficient heating time 18 min. at 99°C), 30 min. incubation of the primary Ab and Titan Super (TT-0805) as detection system.

rmAb clone **644G5A4,** product no. **PA194**, Abcarta, Abcarta FAIP-48T:
One protocol with an optimal result was based on HIER using Abcarta-EDTA/ER2 pH 9 Buffer (efficient heating time 20 min. at 100°C), 32 min. incubation of the primary Ab and Abcarta-HRP Polymer (PS300) 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 MSH2 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb FE11 **IR085**	94% (15/16)	69% (11/16)	100% (10/10)	70% (7/10)
Dako Omnis mAb FE11 **GA085**	100% (35/35)	46% (16/35)	100% (13/13)	54% (7/13)
Leica BOND III/MAX mAb 79H11 **PA0989**	100% (13/13)	92% (12/13)	90% (9/10)	60% (6/10)
VMS Ultra mAb G219-1129 **760-5093**	100% (33/33)	79% (26/33)	93% (106/114)	70% (80/114)

* 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 are integrated.

Comments
In this assessment and in concordance with previous NordiQC MSH2 assessments, the prevalent feature of an insufficient result was a too weak or false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 88% (30/34) of the insufficient results. The remaining 12% (4/34) of insufficient results were characterized by a poor signal-to-noise ratio, false positive staining reactions and/or unspecific granular reaction pattern primarily due to the use of OptiView with amplification (Ventana/Roche), compromising the interpretation of the specific signals.

Virtually all laboratories were able to demonstrate MSH2 in cells with a high-level antigen expression as proliferating germinal centre B-cells in the tonsil, basal epithelial cells of the appendix and neoplastic cells in the MSS colon adenocarcinoma (tissue core no. 3). Demonstration of MSH2 in low-level antigen expressing cells as mantle zone B-cells, smooth muscle cells and stromal/lymphatic cells in the two MMRd colon adenocarcinomas (tissue core no. 4 and 5) was more challenging requiring an optimally calibrated protocol. In this context, it has to be emphasized that identification of loss of MSH2 expression in tumors is characterized by a negative nuclear staining reaction of the neoplastic cells. Consequently, it is critical that normal cells within and around the neoplastic process show a distinct positive nuclear staining reaction, serving as reliable internal positive tissue control.

Only 12% (41/350) of the laboratories used antibodies as concentrated formats within LD assays for MSH2. Compared to the performance of the RTU formats, the pass rate was low as only 73% (30/41) were assessed as sufficient of which 46% (19/41) were optimal (see Table 1). The mAb clones FE11 and G219-1129 were the most widely used antibodies, both providing optimal results within LD assays, 40% (4/10) and 37% (8/22), respectively. As shown in Table 2, the mAb clone FE11 gave optimal result on the Omnis (Dako/Agilent) and the Bond (Leica Biosystems) platforms, whereas the mAb clone G219-1129 provided optimal results on the Benchmark Ultra (Ventana/Roche) and the Bond (Leica Biosystems) instruments. For both clones, virtually all protocols assessed as optimal were founded on efficient HIER in an alkaline buffer (12/12), the primary antibody was carefully calibrated in the dilution range of 1:10-100 (11/12) in combination with a sensitive 3-step detection system (12/12) e.g., EnVision Flex+(Dako/Agilent), Bond Refine (Leica Biosystems) or OptiView (Ventana/Roche).The prevalent causes for an insufficient staining results was use of too diluted antibody e.g., for mAb clone G219-1129 the average dilution factor (ADF) for optimal performance was 1:131 (range 1:50-400) whereas for protocols providing insufficient results, the ADF was 1:344 (range 1:100-1000). In addition, insufficient results were also frequently obtained in combination with other low analytic sensitive parameters as inefficient HIER in acidic buffer and/or use of a 2-step multimer/polymer based detection system.

88% (309/350) of the laboratories used a RTU format for the demonstration of MSH2. This is a significant increase compared to the three previous MSH2 assessments in 2014, 2017 and 2019, in which RTU formats were used by 60%, 69% and 78% of the participants, respectively. As shown in Table 3, the RTU systems GA085 based on the mAb clone FE11 (Omnis, Dako/Agilent), PA0989 based on the mAb clone 79H11 (Bond III/MAX, Leica Biosystems) and 760-5093 based on the mAb clone G219-1129 (Benchmark Ultra, Ventana/Roche) overall provided superior results using vendor recommended protocol settings on the fully automated platforms. Grouped together, all results (81/81) based on these three RTU systems applied as "plug-and-play" were assessed as sufficient and for the RTU system PA0989 (Leica Biosystems) a remarkable proportion of 92% (12/13) of optimal results was obtained.

As observed in the previous run 67 e.g., p53, an unexpected excessive background staining was seen in relation to staining on the Omnis platform (Dako/Agilent). The same unwanted reaction pattern was also observed for MSH2 in this run 68, typically applying the RTU product GA085 (or IR085) based on the mAb clone FE11 on the Omnis. The primary cause for this problem is most likely related to poor lots of the HRP Envision Flex reagents (Dako/Agilent) that has been on the marked for at least 6 months (2022/2023). In total and applying both vendor and laboratory modified protocol settings 69% (33/48) of the protocols based on the Dako/Agilent RTU system GA085, were identified to have a less successfull staining performance that could be related to poor HRP-Envision Flex lots. Of significant importance 52% (17/33) of these assays were downgraded (from optimal to good) due to too excessive background staining compromising the interpretation of the specific signal for MSH2. One protocol displaying excessive background staining simultaneously gave a too weak staining reaction. This aberrant staining pattern is not only restricted to the RTU format GA085 for MSH2 and laboratories are advised to perform vigorous maintenance on the Omnis instruments to minimize the problem. Dako/Agilent is working to solve this problem and will hopefully soon be launching new and better HRP-Envision Flex reagents. The problem with the HRP-Envision Flex reagents is illustrated in Figs. 5a – 5b.

For the Dako/Agilent RTU system IR085 also based on the mAb clone FE11 and developed for the semi-automated platform Autostainer (Dako/Agilent), the pass rate was 94% (15/16) using vendor recommended protocol settings – 69% (11/16) being optimal. A significant proportion of laboratories used this RTU product off-label and 48% (24/50) of the protocols were applied on non-validated platforms as Omnis, Benchmark XT and BOND III/MAX. For participants using this product on the Omnis, the pass rate was 89% (16/18) – 61% (11/18) being optimal. In general, a RTU product should be avoided if not been thoroughly validated to the platform in use. Alternative, and as seen in this assessment, laboratories are encouraged to substitute the non-validated RTU formats with the vendor validated RTU systems performing very well in this assessment.

The most widely used RTU system for demonstration of MSH2 was 760-5093 (Ventana/Roche) based on the mAb clone G219-1129. As mentioned above, and using vendor recommended protocol settings, the proportion of sufficient and optimal results was high, 100% (33/33) and 79% (26/33), respectively. These protocol settings are based on relative short incubation time in the primary antibody (12 min.), HIER in CC1 for 40 min. at 100°C and OptiView as the detection system. For participants modifying the protocol settings and especially prolonging incubation time in primary Ab (≥32 min.) in combination with prolonging HIER time in CC1 (>40 min) and using OptiView with or without amplification, the proportion of optimal results decreased significantly to 60% (15/25). Typically, increased background staining, granular deposit of the amplification reagents and focal nuclear reaction was seen in the MSH2 MMRd colon adenocarcinomas.
As shown in Table 1, and using the RTU format 760-5093 based on laboratory modified protocol settings, 5% (8/152) of the protocols were assessed as insufficient - typically using assays providing low analytical sensitivity as e.g., too short HIER time in CC1 in combination with the low sensitive detection system UltraView.

For the RTU systems from the three above mentioned major vendors, laboratory modified protocol settings could also produce a significant proportion of sufficient and optimal results, although performance was slightly inferior compared to vendor recommended protocol settings for some of the RTU systems (see Table 3).

Fourteen laboratories used the RTU format 286M-17/18 (Cell marque) also based on the mAb clone G219-1129, providing a pass rate of 86% (12/14) of which 43% (6/14) were giving an optimal mark. Most laboratories (11/14) used the RTU product on the Benchmark platforms (Ventana/Roche) and 91% (10/11) gave a sufficient result – 55% (6/11) being optimal.

This was the fifth assessment of MSH2 in NordiQC (see Graph 1). The pass rate increased significantly compared to results obtained in the previous run 57 (2019) for MSH2. In this assessment, the RTU systems/formats gave superior results compared to LD-assays (concentrates), providing a pass rate of 93% (286/309) and 73% (30/41), respectively. This result, and considering the high amount of laboratories using a robust RTU product (88%, 309/350), accounts for the overall improvement in performance seen in this assessment run 68 for MSH2.
Importantly, and for laboratories struggling with the protocols for MSH2, the assay should stain according to the expected antigen level of the recommended control materials (see below).

Controls
Tonsil is recommendable as external positive tissue control for MSH2 and to monitor the IHC test reproducibility with focus on the level of analytical sensitivity. 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.
MMRd colon adenocarcinoma with loss of MSH2 expression could be included as external negative tissue control in which no nuclear staining reaction should be seen in the neoplastic cells, whereas a distinct nuclear staining reaction must be seen in all stromal cells.

For IHC for Mismatch Repair proteins (MMR) as MSH2 it has to be emphasized that internal positive tissue controls being 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 EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

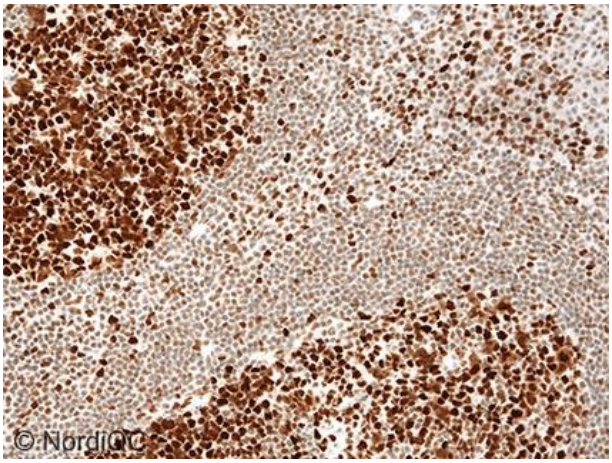


Fig. 1a (x200)
Optimal MSH2 staining of the tonsil using the mAb clone G219-1129, optimally calibrated (1:100), efficient HIER in an alkaline buffer (CC1) and a 3-step multimer based detection system (OptiView) on the Benchmark Ultra platform (Ventana/Roche).
Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction.
Same protocol used in Figs. 2a - 4a.

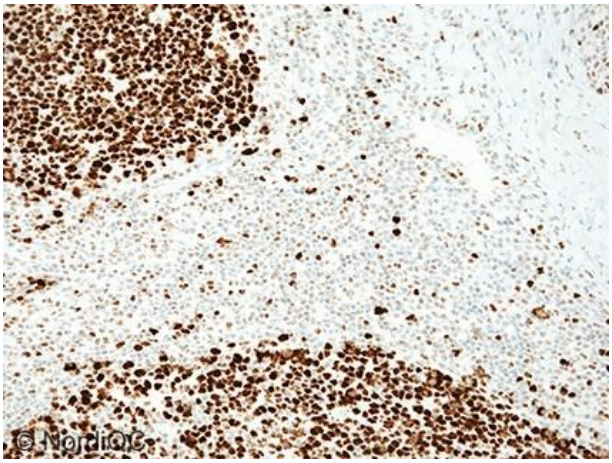


Fig. 1b (x200)
Insufficient MSH2 staining of the tonsil using the mAb clone G219-1129 on the Benchmark Ultra, too diluted (1:600), HIER in CC1 and OptiView with amplification as detection system. Same protocol used in Figs. 2b – 4b.
Only dispersed mantle zone B-cells are demonstrated and staining intensity is too weak although germinal centre B-cells display a strong nuclear staining reaction.
This reaction pattern is a well-known problem using tyramide amplification and typically caused by unbalanced levels of the target protein e.g., low versus high expressors and especially, if the protocol is not well calibrated as in this case. Consequently, the assay is not fit-for-purpose, identifying MSH2 MMRd colon adenocarcinomas – compare Figs. 1a - 4b.

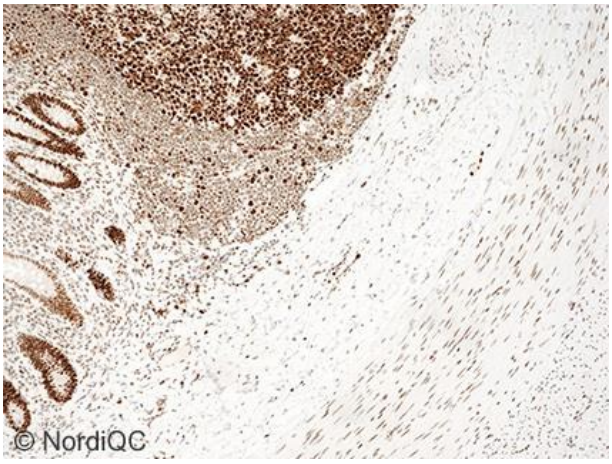


Fig. 2a (x100)
Optimal MSH2 staining of the appendix using same protocol as in Fig. 1a.
Virtually all stromal and epithelial cells show a distinct nuclear staining reaction. Importantly, the smooth muscle cells of lamina muscularis propria must display a weak to moderate, but distinct nuclear staining intensity.
The protocol provided a high signal-to-noise ratio and no background staining is seen.

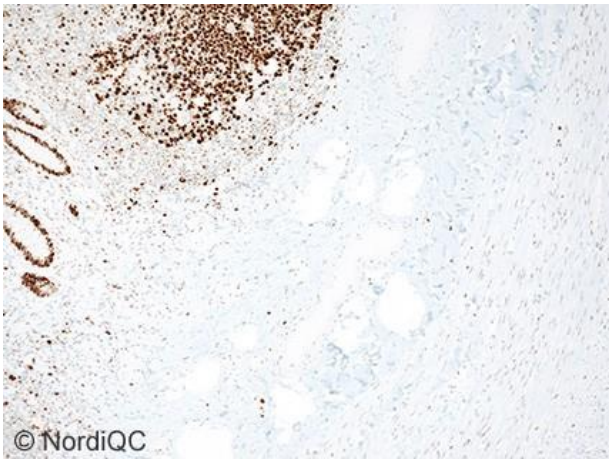


Fig. 2b (x100)
Insufficient staining reaction for MSH2 of the appendix using same protocol as Fig 1.b.
The proportion of positive cells and the staining intensity is significantly reduced compared to the expected result shown in Fig. 2a. A significant proportion of the smooth muscle cells in lamina muscularis propria are false negative or only faintly demonstrated.

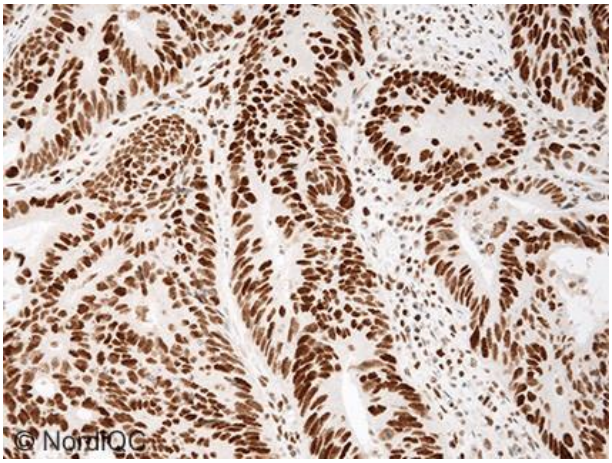


Fig. 3a (x200)
Optimal MSH2 staining of the MSS colon adenocarcinoma, tissue core no. 3, using same protocol as in Figs. 1a - 2a.
All neoplastic cells are positive and essentially, all stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

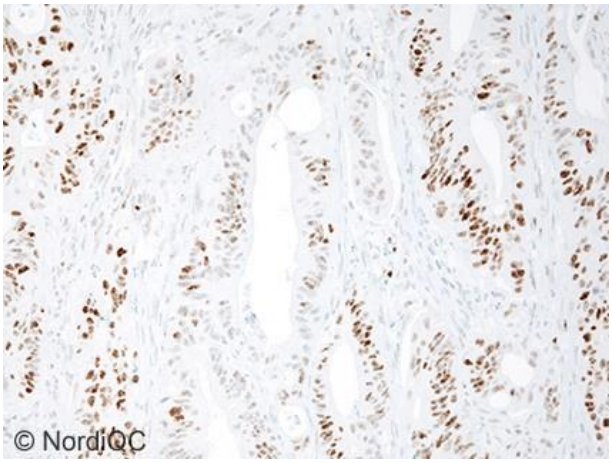


Fig. 3b (x200)
Insufficient MSH2 staining of the MSS colon adenocarcinoma, tissue core no. 3, using same protocol as in Figs. 1b - 2b.
The neoplastic cells, show unexpected partial loss of MSH2 expression and virtually all stromal cells (serving as internal control) are false negative – compare with Fig 3a. Interpretation is challenging, and the outcome of this deviating staining pattern is subsequently illustrated in Fig. 4 b.

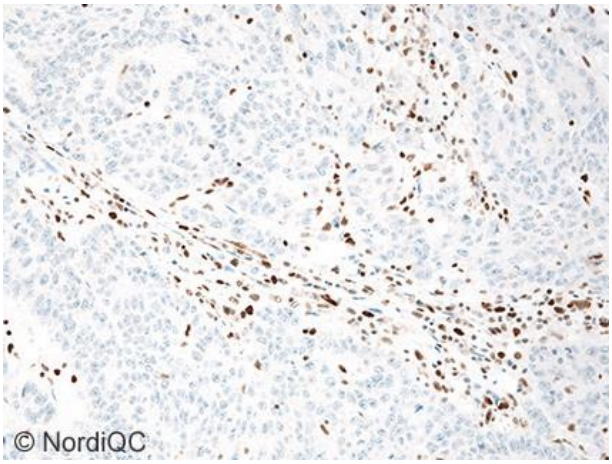


Fig. 4a (x200)
Optimal MSH2 staining of the MMRd colon adenocarcinoma, tissue core no. 5, using the same protocol as in Figs. 1a – 3a.
All neoplastic cells show loss of MSH2 and the stromal cells (internal control) show as expected a distinct nuclear staining reaction. The internal control, e.g. stromal and lymphatic cells, showing a distinct staining intensity of the nuclei`s in proximity to neoplastic tumor cells is of diagnostic importance – otherwise it might be difficult to categorize MMRd colon adenocarcinomas correctly as shown in Fig. 4b.

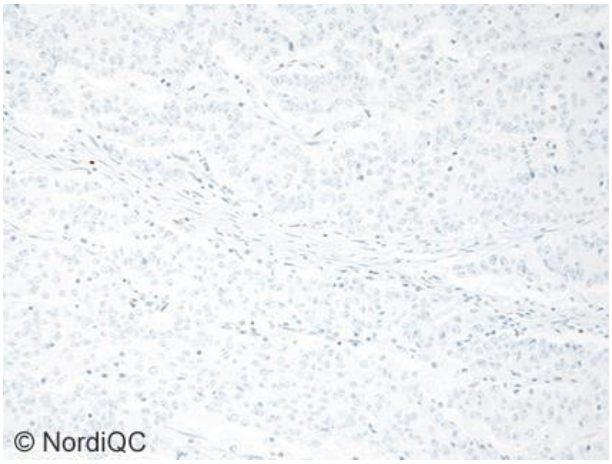


Fig. 4b (x200)
Insufficient MSH2 staining of the MMRd colon adenocarcinoma, tissue core no. 5, using same protocol as in Figs 1b – 3b.
MMR status cannot reliably be interpreted as virtually all stromal and lymphatic cells (internal control) are false negative (very few dispersed cells show a faint staining reaction). In this case, loss of MSH2 expression is difficult to identify due to lack of staining in the internal tissue control - compare with Fig. 4a.

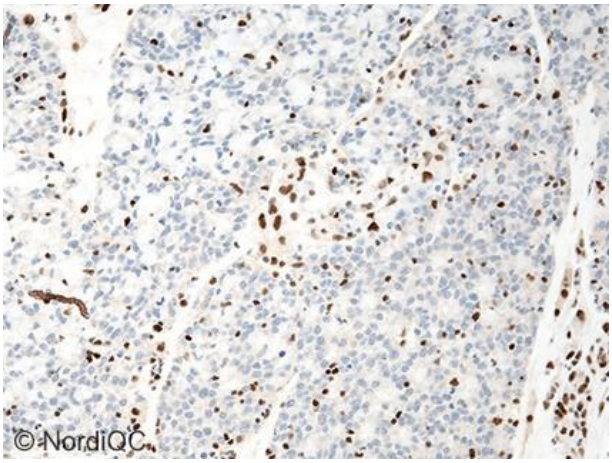


Fig. 5a (x200)
Optimal MSH2 staining of the MMRd colon adenocarcinoma, tissue core no. 4, using the Dako/Agilent RTU system GA085 (Omnis, Dako/Agilent) based on the mAb clone FE11 following the vendor recommended protocol settings including use of EnVision Flex with Dual Linkers as detection system.
The neoplastic cells show as expected loss of MSH2 expression and stromal cells display a distinct nuclear staining reaction serving as internal positive tissue control. It was observed that the HRP-Envision Flex reagent could cause problem with excessive background staining and thus, provided a reduced signal-to-noise ratio as shown in Fig. 5b.

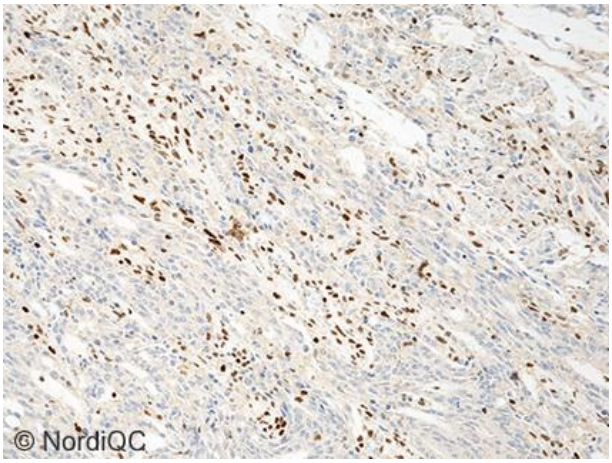


Fig. 5b (x200)
MSH2 staining reaction of the MMRd colon adenocarcinoma, tissue core no. 4, using the Dako/Agilent RTU format GA085 (Omnis, Dako/Agilent) based on the mAb clone FE11 and with protocol settings based on the "less sensitive" EnVision Flex with only the mouse linker as detection system.
Although the protocol theoretically should display a less analytical sensitivity, the cytoplasmic staining reaction in the neoplastic cells complicates the interpretation and thus, was downgraded to good. The problem is likely related to poor HRP-Envision Flex reagents and a significant proportion of protocols gave this aberrant background staining (see description above under comments) - compare with Fig. 5a.

MB/LE/SN 13.06.2023