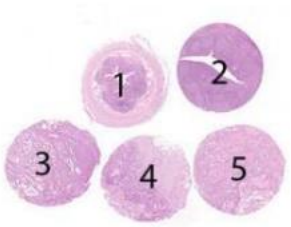
Assessment Run 72 2024

Mismatch repair protein MSH6 (MSH6)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for MSH6 status in colon and endometrial adenocarcinomas. Loss of MSH6 function due to gene mutation or epigenetic changes is characterized by absence of nuclear expression in neoplastic cells, whereas intact nuclear MSH6 expression indicates normal MSH6 function and no gene mutations.

Material
The slide to be stained for MSH6 comprised:



1. Appendix 2. Tonsil 3. Endometrial adenocarcinoma with loss of MSH6 expression, 4. Colon adenocarcinoma with loss of MSH6 expression, 5. Colon adenocarcinoma with normal MSH6 expression

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing MSH6 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, distinct nuclear staining reaction of the germinal center B-cells in the tonsil
- A moderate to strong, distinct nuclear staining reaction of virtually all neoplastic cells and a distinct nuclear staining reaction in the vast majority of other cells (stromal cells, lymphocytes etc) in the colon adenocarcinoma no. 5.
- No nuclear staining reaction of the neoplastic cells in the endometrial and colon adenocarcinomas no. 3* and 4, but a distinct nuclear staining reaction in the vast majority of other cells (stromal cells, lymphocytes etc).

A general weak cytoplasmic staining reaction in cells with coexisting nuclear staining reaction was accepted.

*In this assessment an unexpected cross-reaction with the epithelial cells in the endometrial adenocarcinoma was downgraded, due to interpretational challenges especially in the diagnostic read-out of MSH6 loss. This applied only for rmAb clone SP93 Abs both as concentrated and Ready-to-use formats. The severity of the reaction was both related to the protocols applied and the heterogeneous expression level of the endometrial tumor in the different TMA blocks.

KEY POINTS FOR MSH6 IMMUNOASSAYS
- The mAb clone **EP49** was used by 49% of all participants.
- RTUs were used by 85% of all participants.
- RTUs developed for Autostainer, Omnis, BOND and Benchmark platforms gave superior results applying vendor recommended protocol settings.
- The performance of the mAb clone 44, was less successful.
- Tonsil is recommendable as external positive tissue control to evaluate the analytical sensitivity of MSH6 IHC assays.
- Stromal cells in diagnostic cases must be used to evaluate an adequate MSH6 IHC assay.

Participation

Number of laboratories registered for MSH6, run 72	401
Number of laboratories returning slides	379 (95%)

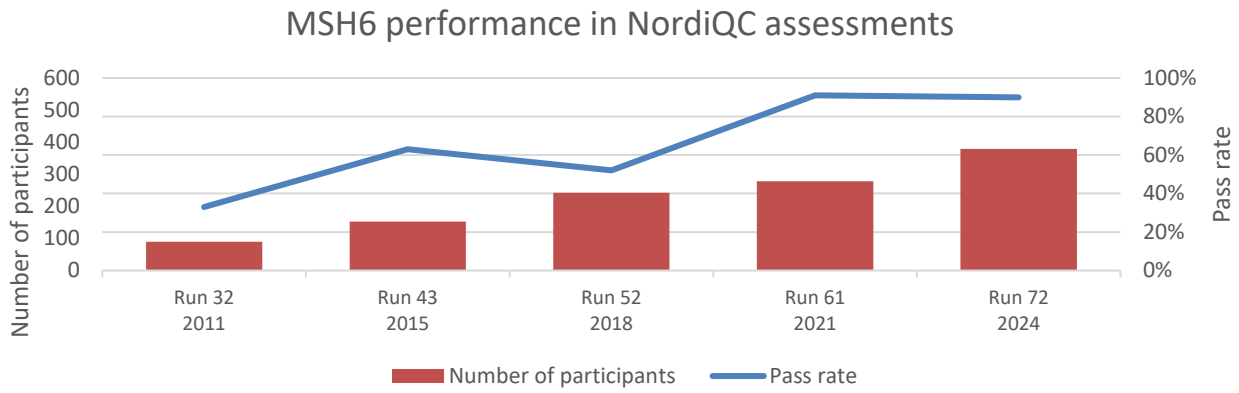
All slides returned after the assessment were assessed and participants received advices if the result was insufficient - data from these outcomes were not included in this report.

Results
379 laboratories participated in this assessment. 342 (90%) of these achieved a sufficient mark (optimal or good). Table 1a-1c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining were:
- Use of less successful clones
- Too low concentration of the primary antibody

Performance history
This was the fifth NordiQC assessment of MSH6. The pass-rate was almost identical compared to the previous runs (see Graph 1).

Graph 1. **Proportion of sufficient results for MSH6 in the fifth NordiQC runs performed**



Controls
Tonsil was found to be a recommendable positive tissue control for MSH6. 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 center B-cells.
Colon adenocarcinoma with loss of MSH6 expression is recommended as negative tissue control. No nuclear staining reaction should be seen in the neoplastic cells, whereas a nuclear staining reaction must be seen in most stromal cells serving as internal positive tissue control. The on-slide tissue controls for MSH6 and MMR proteins will allow to compare inter-assay reproducibility, whereas stromal cells in diagnostic cases will provide information of tissue quality and preservation of the target MMR protein. If the stromal cells are negative this can indicate inappropriate tissue processing as too short or delayed formalin fixation or non-sufficient staining protocol.

Conclusion
Optimal staining results could be obtained with many Abs as the rabbit monoclonal antibodies (rmAb) clones **EP49, EPR3945, BSR100** and **SP93.** Irrespective of the clone applied, efficient HIER in an alkaline buffer and use of a sensitive and specific polymer/multimer based detection system gave the highest proportion of optimal results. The concentration of the primary antibody must be carefully calibrated. The concentrated format of the rmAb clone **EP49** provided optimal staining results on the four main stainer platforms - Omnis (Dako/Agilent), Autostainer (Dako/Agilent), Bond (Leica Biosystems) and BenchMark (Ventana/Roche).
The access to several high quality RTU systems for MSH6 was instrumental for the high pass rate in this run where the vendor recommended protocol settings provided the highest pass-rates on all of the four main IHC platforms.

Table 1a. **Overall results for MSH6, run 72**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	57	35	9	7	6	77%	61%
Ready-To-Use antibodies	322	156	142	22	2	93%	48%
Total	379	191	151	29	8		
Proportion		50%	40%	8%	2%	90%	

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

Table 1b. **Concentrated antibodies and assessment marks for MSH6, run 72**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EP49**	16 13 3 1 1	Dako/Agilent Epitomics Cell Marque BioSB Master Diagnostica	25	6	3	-	91%	74%
mAb clone **44**	3 2 1	Cell Marque Diagnostic BioSystems BD Bioscience	-	-	2	4	-	-
rmAb clone **EPR3945**	5	Abcam	4	-	1	-	80%	80%
rmAb clone **BSR100**	3	Nordic Biosite	3	-	-	-	-	-
rmAb clone **SP93**	2 1	Cell Marque Zytomed Systems	-	2	-	1	-	-
mAb clone **PU29**	2	Leica Biosystems	-	-	1	1	-	-
rmAb clone **ZR342**	1	Zeta Corporation	-	1	-	-	-	-
rmAb clone **QR011**	1	Quartett	1	-	-	-	-	-
rmAb clone **IHC026**	1	GenomeMe	1	-	-	-	-	-
Other clone **24B2-E8**	1	Wondfo	1	-	-	-	-	-
Total	55		35	9	7	6		
Proportion			61%	16%	12%	11%	77%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for MSH6, run 72**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **SP93 760-5092**[3]	78	Ventana/Roche	20	57	1	-	99%	26%
rmAb clone **SP93 760-5092**[4]	82	Ventana/Roche	13	57	12	-	85%	16%
rmAb clone **EP49 GA086**[3]	41	Dako/Agilent	38	3	-	-	100%	93%
rmAb clone **EP49 GA086**[4]	32	Dako/Agilent	24	4	4	-	88%	75%
rmAb clone **EP49 IR086**[3]	12	Dako/Agilent	11	1	-	-	100%	92%
rmAb clone **EP49 IR086**[4]	33	Dako/Agilent	28	3	2	-	94%	85%
rmAb clone **EP49 PA0990**[3]	15	Leica Biosystems	8	5	2	-	87%	53%
rmAb clone **EP49 PA0990**[4]	14	Leica Biosystems	7	7	-	-	100%	50%
rmAb clone **EP49 8326-C010**	3	Sakura Finetek	2	1	-	-	-	-
mAb clone **BC19 API3115H**	1	Biocare Medical	-	1	-	-	-	-
rmAb clone **EP49 MAD-000635QD**	1	Master Diagnostica	-	1	-	-	-	-
rmAb clone **EP49 RMPD045**	1	Diagnostic Biosystems	1	-	-	-	-	-
rmAb clone **EP49 GT219502**	1	Gene Tech	1	-	-	-	-	-
mAb clone **MX056 MAB-0831**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone **SP93 BFM-0042**	1	Bioin Biotechnology	-	-	1	-	-	-
rmAb clone **SP93 287R-27/28**	1	Cell Marque	-	1	-	-	-	-
rmAb clone **44 287M-10/17/18**	1	Cell Marque	-	-	-	1	-	-
rmAb clone **44 PM265AA**	1	BioCare	-	-	-	1	-	-
rmAb clone **BP6007 I10102E-05**	1	Biolynx Biotechnology	1	-	-	-	-	-
mAb clone **821G2C1 PA195**	1	Abcarta	1	-	-	-	-	-
mAb clone **ZM99 Z2409MP**	1	Zeta Corporation	-	1	-	-	-	-
Total	322		156	142	22	2		
Proportion			48%	44%	7%	1%	93%	

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 MSH6, Run 72

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

rmAb clone **EP49**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (2/2), TRS pH 9 (Dako/Agilent) (3/5), Cell Conditioning 1 (CC1, Ventana/Roche) (10/14), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (7/9), Bond-Prime Epitope Retrieval Solution 2 (BPERS2, Leica Biosystems) (3/3) as retrieval

buffer. The rmAb was typically diluted in the range of 1:25-1:250 depending on the total sensitivity of the protocol employed. Using these protocol settings, 30 of 32 (94%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EPR3945**: Protocols with optimal results were all based on HIER using TRS pH 9 (Dako/Agilent) (1/1), CC1 (Ventana/Roche) (2/3) and BERS 2 (Leica Biosystems) (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:200-1:1,000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 4 of 5 (80%) laboratories produced an optimal staining result.

Table 2. **Proportion of optimal results for MSH6 for the most commonly used antibody concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	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
rmAb clone **EP49**	2/2	-	3/5 (60%)	-	10/14 (71%)	-	10/12 (83%)	0/1
rmAb clone **EPR3945**	-	-	1/1	-	2/3	-	1/1	-

* 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)
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra Plus
3) Bond III, Prime, Max

Ready-To-Use antibodies and corresponding systems
rmAb clone **SP93**, product no. **760-5092**, Ventana/Roche, Ventana Benchmark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time for 48-64 min. at 100°C), 8-16 min. incubation at 36°C of the primary Ab and UltraView (760-500) or OptiView (760-700) with or without amplification (760-080/760-099) as detection system. Using these protocol settings 92 of 95 (97%) laboratories produced a sufficient staining result (optimal or good).
2 laboratories used product no 760-5092 on other platforms. These were not included in the description above.

rmAb clone **EP49**, product no. **GA086**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER in TRS pH 9 (efficient heating time 20-30 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+/FLEX++ (GV8000/GV823/GV809) as detection system. Using these protocol settings 60 of 62 (97%) laboratories produced a sufficient staining result (optimal or good).
6 laboratories used product no GA086 on other platforms. These were not included in the description above.

rmAb clone **EP49**, product no. **IR086/IS086**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 95-97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system. Using these protocol settings 23 of 23 (100%) laboratories produced a sufficient staining result (optimal or good).
22 laboratories used product no IR086/IS086 on other platforms. These were not included in the description above.

rmAb clone **EP49**, product no. **PA0990**, Leica Biosystems, Bond III, Bond Prime:
Protocols with optimal results were typically based on HIER in BERS2 (efficient heating time 10-20 min. at 100°C), 15-30 min. incubation of the primary Ab and Bond Refine (DS9800) as detection system. Using these protocol settings 21 of 23 (91%) laboratories produced a sufficient staining result (optimal or good).
1 laboratory used product no PA0990 on another platform. This was not included in the description above.

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 accordingly 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 3. **Proportion of sufficient and optimal results for MSH6 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS BenchMark rmAb clone **SP93, 760-5092**	99% (77/78)	26% (20/78)	86% (68/79)	16% (13/79)
Dako Omnis rmAb clone **EP49, GA086**	100% (41/41)	93% (38/41)	92% (23/25)	80% (20/25)
Dako AS rmAb clone **EP49, IR086/IS086**	100% (12/12)	92% (11/12)	100% (11/11)	100% (11/11)
Leica Biosystems rmAb clone **EP49, PA0990**	87% (13/15)	53% (8/15)	100% (13/13)	46% (6/13)

* 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 of MSH6, the prevalent feature of an insufficient result was either a too weak or false negative staining reaction of cells expected to be demonstrated, which was seen in 68% (25/37) of the insufficient results. The majority of the remaining insufficient results (12/37) displayed an aberrant membranous pattern in the endometrial adenocarcinoma and/or a general granular staining interfering with the interpretation. Generally, most laboratories could demonstrate MSH6 in cells with high-level antigen expression as proliferating germinal center B-cells in the tonsil, basal epithelial cells of the appendix and neoplastic cells in the colon adenocarcinoma with normal MSH6 expression. Demonstration of MSH6 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 MSH6 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.

15% (57 of 379) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for MSH6. Optimal staining result could be obtained with the rmAbs clones EP49, EPR3945, BSR100, QR011 and IHC026 (see Table 1b). Irrespective of the clone applied, careful calibration of the titre, efficient HIER, preferably at high pH and 3-step polymer/multimer based detection system were the main protocol prerequisites for optimal results.
The rmAb clone EP49 was the most widely used Ab for demonstration of MSH6 and provided a high proportion of sufficient staining results. Optimal results could be obtained on all four main IHC systems from Dako/Agilent, Leica Biosystems and Ventana/Roche (see Table 2). It should be noted that the titer on the Bond platforms demanded a higher concentration (below 1:100) to produce optimal results due to the limitation with the Bond Refine detection system only acting as a 2-layer system when using rabbit antibodies.

In contrast, no optimal results were registered with the mAbs clones 44, PU29 and ZR342 or the rmAb clone SP93

Ready-To-Use (RTU) antibodies was used by 85% (322 of 379) of the laboratories. The use of RTU products has been steadily increasing from 53% in 2015 and through out the last couple of runs.

The RTU system from Ventana/Roche based on rmAb clone SP93 (760-5093) was the most widely used system and obtained high pass-rates both following the vendor recommended protocol settings or modifying the protocol of 99% and 86% respectively (see Table 3). However, in this run it was observed that the SP93 clone both as RTU products and concentrated formats frequently produced an aberrant membranous staining in the endometrial adenocarcinoma which was downgraded, due to interpretational challenges especially as the endometrial adenocarcinoma was one of the neoplastic tissues displaying loss of the MSH6 expression (see figs 5a-5b). After the assessment, NordiQC tested 9 more MSH6 deficient endometrial adenocarcinomas with the SP93 clone using different protocol setups and in 2 cases the membranous reaction was observed concluding this was not a rare and single occurrence. In this context it has to be emphasized, that no aberrant membranous reaction was seen in the endometrial adenocarcinoma included for the assessment with other Abs than clone SP93 and all 10 endometrial adenocarcinomas tested by NordiQC using the rmAb clone EP49 showed the expected nuclear loss of MSH6 without any disturbing membranous staining pattern. At present, no explanation for the aberrant membranous staining reaction has been identified. It is as such not possible to say if the reaction is caused by e.g. a truncated MSH6 protein being expressed due to a MSH6 mutation or related to an unspecific cross reaction with an unknown antigen. However, as the read-out of MSH6 was compromised, it was

decided that the pattern was not compatible with an optimal score and thus downgraded to good if all other tissue cores showed the pattern expected.
The proportion of optimal results was dependent on the TMA used as the adenocarcinoma showed a heterogeneous expression as some areas showed a more prominent and extensive membranous reaction pattern than others. In total 6 different TMA´s was used for the assessment. All 6 TMA's were constructed with same donor material. The aberrant membranous staining reaction in the endometrial adenocarcinoma was the main cause of the very low proportion of optimal results for both the vendor recommended protocol settings and the laboratory modified protocols (26% and 16%, respectively) for the Ventana/Roche 760-5093 RTU system based on clone SP93. The vendor recommended protocol using HIER inCC1 for 64 min. and a primary Ab incubation of 12 min along with OptiView as detection system did in all other cores display an optimal staining reaction.
The most common protocol modifications were related to increased incubation time of primary Ab and/or a decreased time of HIER. Some laboratories applied OptiView Amplification kit, which in 9 cases gave an excessive granular staining reaction, compromising the interpretation (see figs 5b and 6b).

The Dako/Agilent RTU systems based on rmAb clone EP49 IR086/IS086 and GA086 for Autostainer link 48 and Omnis were used by a total of 118 laboratories and provided a pass-rate of 100% when used by the vendor recommended protocol settings and an impressive proportion of optimal results of 92% and 93%, respectively (Table 1C and 3). The recommended protocol for the Omnis RTU is based on a 4-layer detection system, while the Autostainer RTU is based on a 2-layer detection system. 22 laboratories were using the IR086/IS086 product and 6 the GA086 product on other platforms mainly Ventana and Bond platforms with high pass-rates.

The Leica Biosystems RTU product PA0990 also provided a high pass rate using the rmAb clone EP49 both as a vendor recommended and as laboratory modified protocol. The level of optimal protocols was lower than for the Dako/Agilent system where a too weak staining was the main reason for non-optimal results.

This was the fifth assessment of MSH6 in NordiQC (see Graph 1), but the first when both colon and endometrial samples were included. The proportion of sufficient results was 90% which is almost identical to the passrate of 91% in the previous run 61 (2021).
Similar to the previous run, the main parameters contributing to the high pass-rate were related to the access to and widely used robust Abs as clone EP49, EPR3945 and BSR100 on the expense of the unsuccessful mAb clone 44. Also, the extensive use of well performing RTU systems from the main IHC system providers impacted the pass rate positively. In this context both the RTU system from Dako/Agilent and the RTU system from Ventana/Roche both gave a high pass rate of 100% and 99%, respectively, when applied by recommended protocol settings.
However, only 26% optimal results were observed for the Ventana/Roche system due to an unexpected aberrant membranous reaction in the endometrial adenocarcinoma which were only seen for the SP93 clone.
Although some clones seem to be relatively more robust, it is important to conclude that sufficient HIER and careful calibration of the primary antibody concentration together with a sensitive detection system is an important prerequisite for an optimal MSH6 staining reaction.

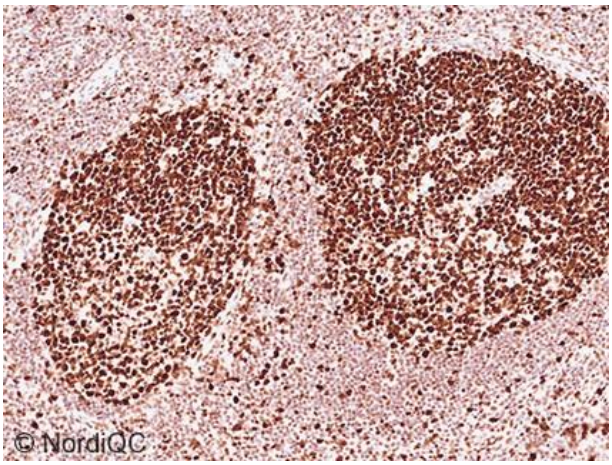


Fig. 1a
Optimal MSH6 staining reaction of the tonsil using the Dako RTU GA086 format based on rmAb clone EP49, using the recommended protocol settings with HIER in an alkaline buffer (TRS pH 9) and a 4-step polymer-based detection system (EnVision Flex with both Rabbit and Mouse Linker). Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction.
Also compare with Figs. 2a - 4a, same protocol.

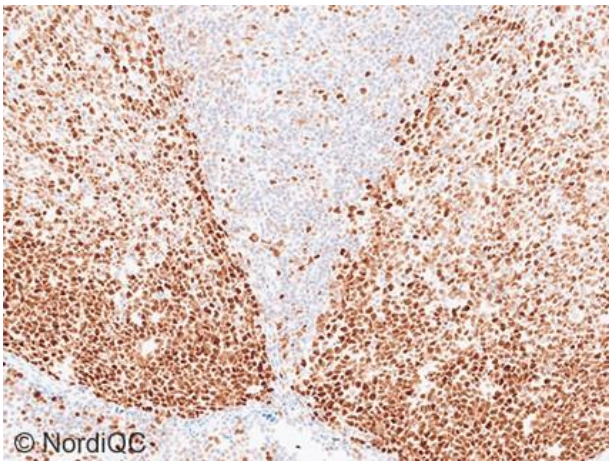


Fig. 1b
Insufficient MSH6 staining reaction of the tonsil using the Dako RTU GA086 format based on rmAb clone EP49, but only with a 2-step polymer-based detection system (EnVision Flex). Only the germinal centre B-cells are distinctively demonstrated, while mantle zone B-cells with low level MSH6 expression virtually are unstained.
Also compare with Figs. 2b - 4b, same protocol.

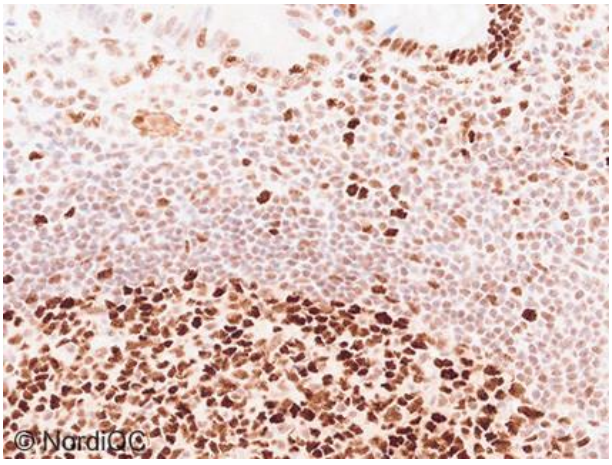


Fig. 2a
Optimal MSH6 staining reaction of the appendix using same protocol as in Fig. 1a. Virtually all cells show an at least weak to strong nuclear staining reaction.
Also compare with Figs. 3a - 4a, same protocol.

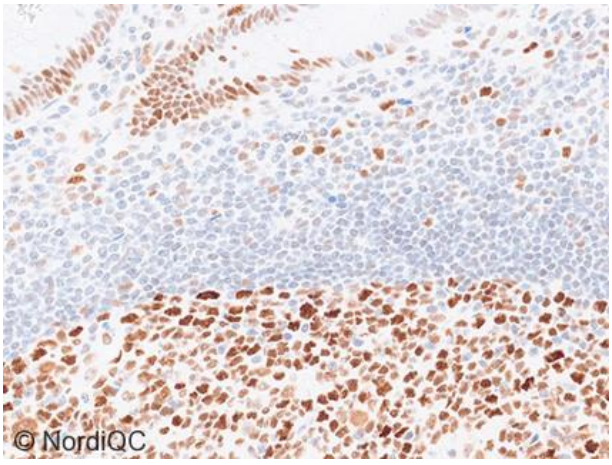


Fig. 2b
Insufficient MSH6 staining reaction of the appendix, using same protocol as in Fig. 1b. Crypt epithelial cells and germinal center B-cells are positive, but many of the lymphocytes are false negative.
Also compare with Fig. 3b - 4b, same protocol.

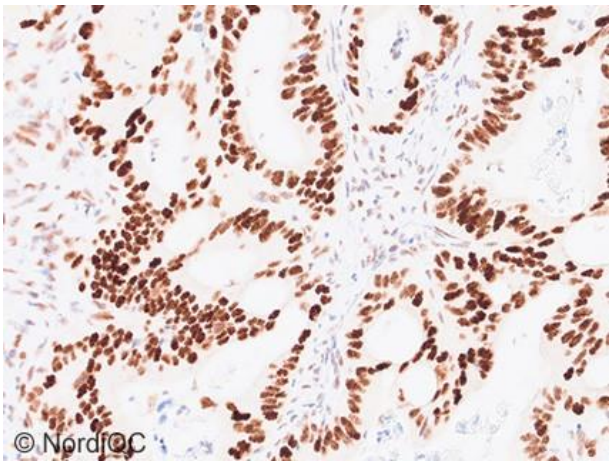


Fig. 3a
Optimal MSH6 staining reaction of the colon adenocarcinoma, tissue core no. 5, with preserved MSH6 expression using same protocol as in Figs. 1a - 2a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. Stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. Also compare with Figs. 4a, same protocol.

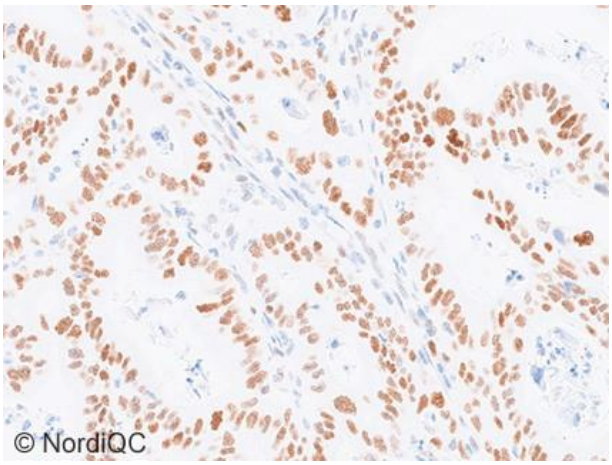


Fig. 3b
MSH6 staining reaction of the colon adenocarcinoma, tissue core no. 5, with preserved MSH6 expression using same protocol as in Figs. 1b - 2b. A weak to moderate staining reaction was seen in the neoplastic cells. The proportion and the intensity of stromal cells demonstrated is reduced compared to the optimal result in Fig. 3a.

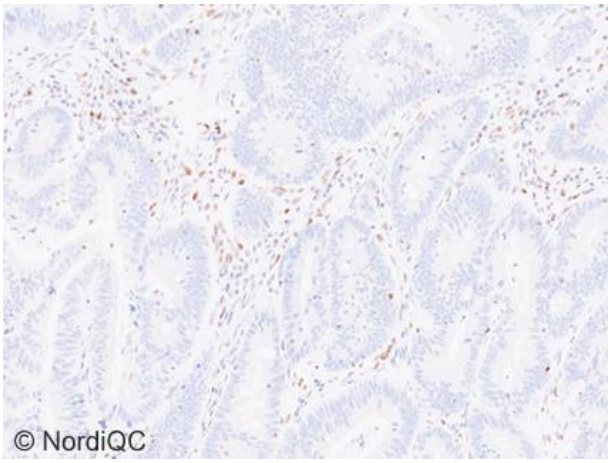


Fig. 4a
Optimal MSH6 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of MSH6 expression using same protocol as in Figs. 1a - 3a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. No background staining is seen.

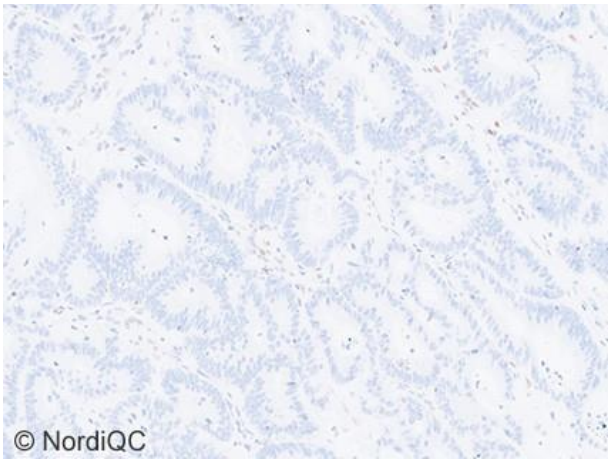


Fig. 4b
Insufficient MSH6 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of MSH6 expression using same protocol as in Figs. 1b – 3b. The neoplastic cells are negative. The proportion and the intensity of stromal cells demonstrated is significantly reduced compared to the expected and optimal result as shown in Fig. 4a.

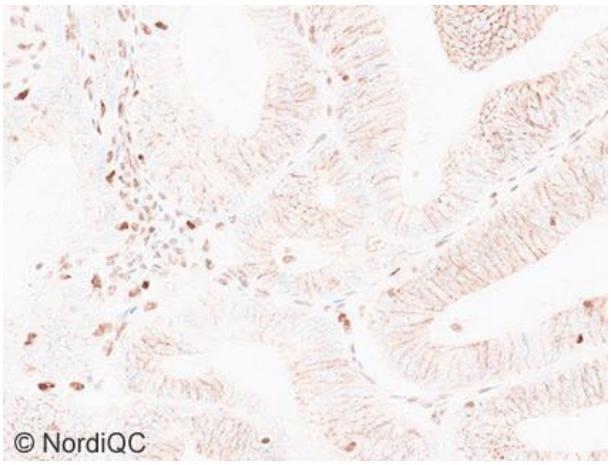


Fig. 5a
Sufficient (scored as Good) MSH6 staining reaction of the endometrial adenocarcinoma, tissue core no. 3, with loss of MSH6 expression using rmAb clone SP93 based 760-5092 RTU system (Ventana/Roche), using the recommended protocol settings, same protocol as in Fig. 6a. The neoplastic cells are negative in the nuclei but display a positive membranous reaction interfering with the interpretation. The stromal cells display a distinct nuclear staining reaction serving as internal positive tissue control.

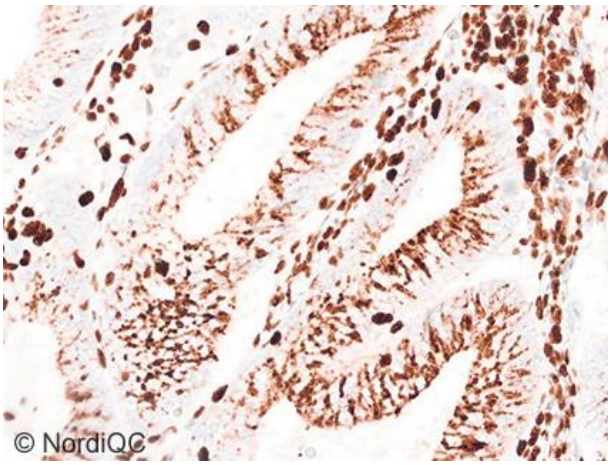


Fig. 5b
Insufficient MSH6 staining reaction of the endometrial adenocarcinoma, tissue core no. 3, with loss of MSH6 expression using rmAb clone SP93 based 760-5092 RTU system (Ventana/Roche), using OptiView with amplification, same protocol as in Fig. 6b. The membrane reaction in the neoplastic cells makes it very difficult to conclude a loss of MSH6, and a diffuse granular reaction pattern was observed in most of the other tissue cores. Same field as 5a.

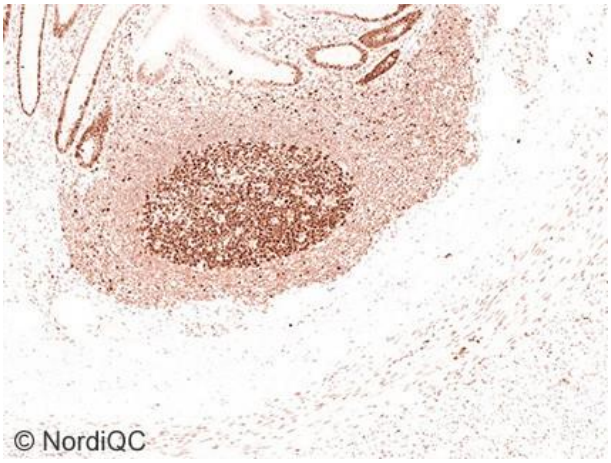


Fig. 6a
Sufficient MSH6 staining reaction of the appendix using the same protocol settings as in Fig. 5a. Virtually all cells show an at least weak to strong nuclear staining reaction.

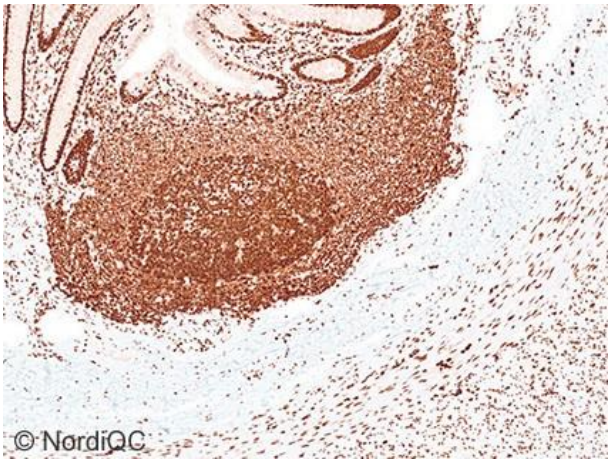


Fig. 6b
MSH6 staining reaction of the appendix, using same insufficient protocol as in Fig. 5b - same field as in Fig. 6a. All cells display a strong nuclear staining reaction and the epithelial cells in the crypts also display a general weak cytoplasmic staining reaction which was accepted in cells with coexisting nuclear staining reaction.

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