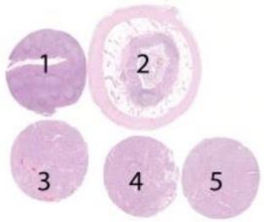
Assessment Run 50 2017

Mismatch Repair Protein MSH2 (MSH2)

Material
The slide to be stained for MSH2 comprised:



1. Tonsil, 2. Appendix, 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 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 centre B-cells in the tonsil.
- A moderate to strong, distinct nuclear staining reaction in virtually all neoplastic cells of the colon adenocarcinoma no. 3.
- No nuclear staining reaction of the neoplastic cells of the colon adenocarcinomas no. 4 and no. 5, but a distinct nuclear staining reaction in the vast majority of other cells (stromal cells, lymphocytes etc.).

A weak cytoplasmic staining reaction was accepted.

Participation

Number of laboratories registered for MSH2, run 50	244
Number of laboratories returning slides	231 (95%)

Results
231 laboratories participated in this assessment. Of these, 182 (79%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks given (see page 2).

The most frequent causes of insufficient staining reaction were:
- Less successful primary antibody
- Too low concentration of the primary antibody
- Insufficient heat induced epitope retrieval (HIER)
- Use of less sensitive detection systems

Performance history
This was the third NordiQC assessment of MSH2. Despite a significant increase in the number of participants, the pass rate was increased compared to run 41, 2014. The reason for this is unclear but could be related to an increase in the proportion of laboratories using the very successful Ready-To-Use systems based on the mAb clones FE11 and G219-1129. A similar increase in the proportion of laboratories using sensitive 3-step polymer/multimer detection systems was also registered and this might likewise have a positive impact on the pass rate.

Table 2. **Proportion of sufficient results for MSH2 in three NordiQC runs performed**

	Run 22 2008	Run 41 2014	Run 50 2017
Participants, n=	51	143	231
Sufficient results	73%	67%	79%

Conclusion
The mAb clones **FE11**, **G219-1129** and **25D12** and the rmAb clone **RED2** could all be used to obtain optimal staining results for MSH2, but clones FE11 and G219-1129 were clearly the most successful clones. Irrespective of the clone applied, HIER in an alkaline buffer and the use of a sensitive and specific 3-step polymer/multimer based detection system gave the highest proportion of optimal results. The

concentration of the primary antibody had to be carefully calibrated to the detection system and the IHC system applied.
The Ready-To-Use systems based on clone FE11 (Dako/Agilent IR085) and clone G219-1129 (Ventana/Cell Marque 760-4265), provided the highest proportion of sufficient and optimal results. 47% (18 of 38) of the laboratories using the Dako/Agilent RTU system IR085 followed the recommended protocol settings and was rewarded with a pass rate of 100%, with 94% being optimal. In contrast only 5% (3 of 70) of the laboratories using the Ventana/Cell Marque RTU system 760-4265 followed the recommended protocol settings and only one laboratory obtained optimal results. Fortunately, a satisfactory high pass rate (91%) and proportion of optimal (64%) was seen for the laboratory modified protocols for the 760-4265 system.

Tonsil is recommendable as positive tissue control for MSH2. Mantle zone B-cells must show an at least weak to moderate nuclear staining reaction, while a moderate to strong nuclear staining reaction must be seen in proliferating germinal centre B-cells. Tumour tissue, e.g. colon adenocarcinoma, with loss of MSH2 expression must be used as negative tissue control, in which no nuclear staining reaction in the neoplastic cells should be seen, whereas a distinct nuclear staining reaction must be seen in stromal cells.

Table 1. **Antibodies and assessment marks for MSH2, run 50**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **25D12**	7 1	Leica/Novocastra Zhangoshan	1	2	3	2	36%	-
mAb clone **FE11**	15 7 6	Dako/Agilent BioCare Millipore/Calbiochem	7	12	9	0	68%	79%
mAb clone **G219-1129**	28 4 1 1 1	Cell Marque BD Biosciences Immunologic Menarini Monosan	10	15	8	2	71%	80%
Ready-To-Use antibodies								
mAb clone **25D12 PA0048**	4	Leica/Novocastra	0	1	2	1	-	-
mAb clone **25D12 PDM179**	1	Diagnostic Biosystems	0	0	1	0	-	-
mAb clone **25D12 MAB-0291**	1	Maixin	0	0	1	0	-	-
mAb clone **FE11 IR085**	55	Dako/Agilent	36	12	5	2	87%	94%
mAb clone **FE11 MSG031**	4	Zytomed Systems	2	0	2	0	-	-
mAb clone **FE11 MAD-000677QD**	2	Master Diagnostica	2	0	0	0	-	-
mAb clone **FE11 PM219**	1	Biocare	0	1	0	0	-	-
mAb clone **G219-1129 760-4265**	70	Ventana/Cell Marque	44	19	5	2	90%	91%
mAb clone **G219-1129 286M-18**	20	Cell Marque	12	4	3	1	80%	-
rmAb clone **RED2 8327-C010**	1	Sakura Finetek	1	0	0	0	-	-
rmAb clone **SP46 AN743-5M**	1	BioGenex	0	1	0	0	-	-
Total	231		115	67	39	10	-	
Proportion			50%	29%	17%	4%	79%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of MSH2, Run 50
The following protocol parameters were central to obtain an optimal staining:

Concentrated antibodies
mAb clone **25D12**: One protocol with an optimal result was based on HIER in water bath using Tris-EDTA / EGTA pH 9 (efficient heating time 15 min. at 97°C), 50 min. incubation of the primary Ab diluted 1:30 and GTVision (Gene Tech; GK6007) as detection system.

mAb clone **FE11**: Protocols with optimal results were all based on HIER using Bond Epitope Retrieval Solution 2 (BERS2; Leica) (3/13)*, Target Retrieval Solution (TRS) High pH (Dako) (2/2), Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (1/5) or Tris-EDTA / EGTA pH 9 (1/2) as retrieval buffer. The mAb was diluted in the range of 1:15-1:100 depending on the total sensitivity of the protocol employed.
Using these protocol settings, 15 of 19 (79%) 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) (5/20) or TRS High pH (Dako) (5/5) as retrieval buffer. The mAb was diluted in the range of 1:25-1:400 depending on the total sensitivity of the protocol employed.
Using these protocol settings, 20 of 25 (80%) laboratories produced an optimal staining result.

Table 3. **Proportion of optimal results for MSH2 for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb clone **FE11**	1/5** (20%)	-	1/1	-	0/5 (0%)	-	3/13 (23%)	-
mAb clone **G219-1129**	0/1	0/1	5/5 (100%)	-	5/20 (25%)	-	0/5 (0%)	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, 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 95-99°C), 20 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) with or without mouse linker (K8021) as detection system.
Using these protocol settings 33 of 35 (94%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **G219-1129,** product no. **760-4265**, Ventana/Cell Marque, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER using Cell Conditioning 1 or Cell Conditioning 2 (efficient heating time 20-64 min.), 8-60 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit ((760-080) or OptiView (760-700) +/- amplification kit (760-099 / 860-099) as detection systems. Using these protocol settings 62 of 68 (91%) laboratories produced a sufficient staining result.

rmAb clone **RED2,** product no. **8327-C010**, Sakura Finetek, Genie:
One protocol with an optimal result was based on HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer (efficient heating time 60 min. at 98°C), 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) as detection system.

Table 4 summarises the proportion of sufficient and optimal marks for the most commonly used RTU systems. The performance was evaluated both as "true" plug-and-play systems performed strictly 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 4. **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**	100% (18/18)	94% (17/18)	85% (17/20)	50% (10/20)
VMS Ultra/XT mAb G219-1129 **760-4265**	67% (2/3)	33% (1/3)	91% (61/67)	64% (43/67)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer integrated.

Comments

In this assessment and in concordance with the 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 86% of the insufficient results (42 of 49 laboratories). The remaining 14% insufficient results were characterized by a poor signal-to-noise ratio and false positive staining reaction compromising interpretation.

The majority of 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 colon adenocarcinoma, tissue core no. 3, with normal MSH2 expression. Demonstration of MSH2 in low-level antigen expressing cells (as resting mantle zone B-cells, smooth muscle cells and stromal cells in the two colon adenocarcinomas) was more challenging and required an optimally calibrated protocol. In this context it has to be emphasized, that identification of loss of MSH2 expression in tumours is characterized by a negative nuclear staining reaction of the neoplastic cells. Consequently, it is of decisive importance that normal cells within and around the neoplastic tissue show a distinct positive nuclear staining reaction, serving as reliable internal positive tissue control (Fig. 1 – Fig. 4).

31% (71 of 231) of the laboratories used Abs as concentrated format within laboratory developed (LD) assays for MSH2. The mAb clones FE11 and G219-1129 were the most widely used antibodies (see Table 1) and could both be used to obtain an optimal staining result. The proportion of sufficient staining results and optimal staining results were similar for the two clones. 71% of the laboratories using the mAb G219-1129 in a LD assay produced sufficient staining results of which 29% were optimal. The results for the mAb clone FE11 in a LD assay were 68% sufficient of which 25% were optimal. For both mAb clone FE11 and mAb clone G219-1129 and in concordance with previous MSH2 assessments, the hallmarks of LD-protocols giving optimal results were use of HIER in an alkaline buffer (pH 8-9) in combination with a 3-step polymer/multimer based detection system as EnVision Flex+ (Dako), OptiView (Ventana) and Refine (Leica).

In the previous MSH2 assessments in 2012, no laboratories using the mAb clone 25D12 achieved sufficient results. In the present assessment the performance was improved, with 3 of 8 laboratories achieving sufficient results, but still the performance of mAb clone 25D12 was inferior to the mAbs clone FE11 and G219-1129.

With mAb clone FE11, optimal results could be obtained on the most widely used IHC platforms, except for the Ventana BenchMark platform (see Table 3). Though the number of laboratories are small, it should be noticed that 4 of the 5 laboratories using the mAb clone FE11 on the Ventana BenchMark got insufficient results indicating, that mAb clone FE11 might be difficult to optimize on this platform (see Fig. 5). With mAb clone G219-1129, optimal results could be obtained on both the Dako Omnis and the Ventana BenchMark platforms, whereas no optimal results were registered on the Dako Autostainer and Leica Bond platforms (see Table 3). Remarkable, all 5 laboratories using mAb G219-1129 on the Dako Omnis achieved optimal results.

69% (160 of 231) of the laboratories used Abs in Ready-To-Use (RTU) formats. This was a significant increase compared to the previous MSH2 assessment in 2012, where 60% of the laboratories used the RTU format. The most widely used RTU systems for MSH2 were the mAb clone G219-1129 based **760-4265** from Ventana/Cell Marque and the mAb clone FE11 based **IR085** from Dako/Agilent. Both provided a higher proportion of sufficient and optimal results compared to laboratory developed protocols using the same clones as concentrates (see Table 1). Optimal results could both be obtained by using the official protocol recommendations given by the two companies for the RTU formats and by laboratory modified protocol settings.

47% of the laboratories using the Dako/Agilent IR085 system followed the recommended protocol settings and all (18 of 18) achieved sufficient results, with 94% optimal (17 of 18). For the laboratory modified

protocol settings the results were inferior. 85% (17 of 20) of the laboratories achieved sufficient results but only 50% (10 of 20) were optimal.
For the Ventana/Cell Marque system 760-4265 recommended protocol settings are provided for both the UltraView (2-step multimer) and OptiView (3-step multimer) detection systems. Only 3 of 70 (4%) laboratories using the 760-4265 system followed the recommended protocol settings. One laboratory follow the recommended OptiView protocol and achieved optimal results, whereas two laboratories followed the UltraView recommendation and both failed to produce optimal results. In contrast 96% (67 of 70) of the laboratories used laboratory modified protocol settings, resulting in 64% (43 of 67) of the laboratories achieving optimal results. 52 laboratories based their modifications on the OptiView protocol and 71% (37 of 52) obtained optimal results, 6 laboratories based their modifications on the UltraView protocol with amplification and 66% were optimal (4 of 6). 8 laboratories modified the basic 2-step UltraView protocol and only 25% (2 of 8) obtained optimal results. The hallmarks of the optimal laboratory modified protocol settings were typically an increase in the incubation time of the primary Ab and extended HIER time in high pH buffer CC1. Consequently, for the OptiView protocols the average Ab incubation time for the laboratories achieving optimal result increased from the recommended 8 min. to 25 min. and for the HIER time an increase from 32 min. in CC1 to an average of 46 min. in CC1 was registered. These data indicate that the recommended protocol settings from Ventana/Cell Marque should be revised.

Controls
Tonsil was found to be a recommendable positive tissue control for MSH2 (see Fig. 1). 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 with loss of MSH2 expression is recommended as negative tissue control for MSH2 (see Figs. 3 and 4). No nuclear staining reaction should be seen in the neoplastic cells, whereas a distinct nuclear staining reaction must be seen in stromal cells.

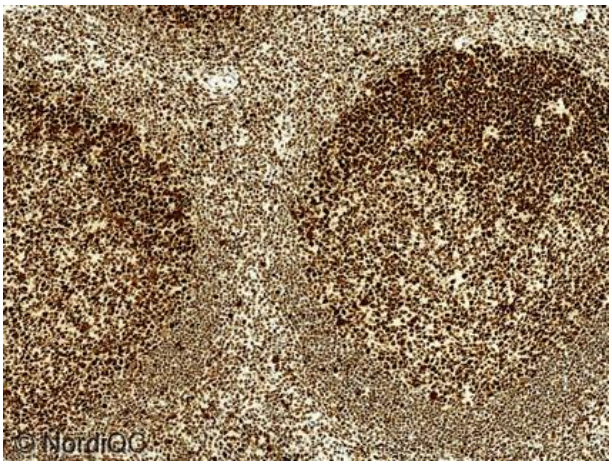


Fig. 1a
Optimal MSH2 staining of the tonsil using the mAb clone FE11, optimally calibrated, HIER in an alkaline buffer and a 3-step polymer based detection system on the Dako Omnis stainer. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Also compare with Figs. 2a - 4a, same protocol.

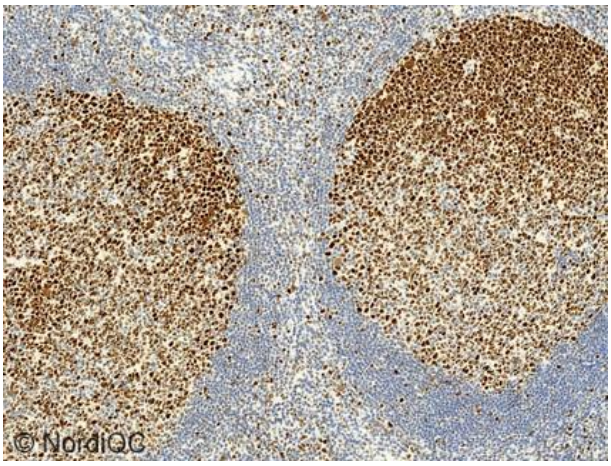


Fig. 1b
Insufficient MSH2 staining of the tonsil using the mAb clone FE11 with similar protocol settings, except for the use of 2-step polymer based detection system on the Dako Omnis stainer - same field as in Fig. 1a. Only the germinal centre B-cells are demonstrated, while mantle zone B-cells expressing low level MSH2 virtually are unstained. Also compare with Figs. 2b - 4b, same protocol.

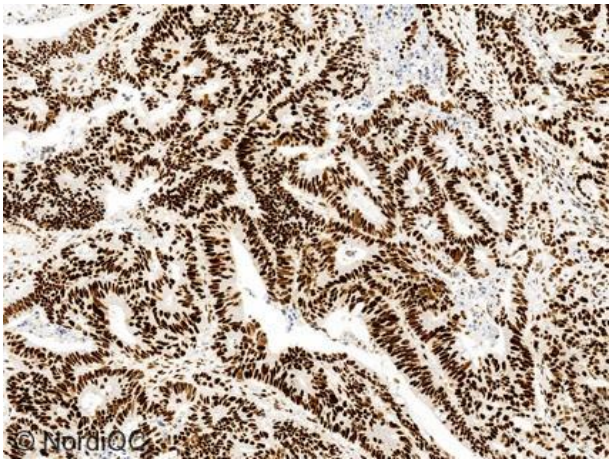


Fig. 2a
Optimal MSH2 staining of the colon adenocarcinoma, tissue core no. 3, with normal MSH2 expression using same protocol as in Fig. 1a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained. No background staining is seen and a distinct nuclear staining reaction in the stromal cells is seen.

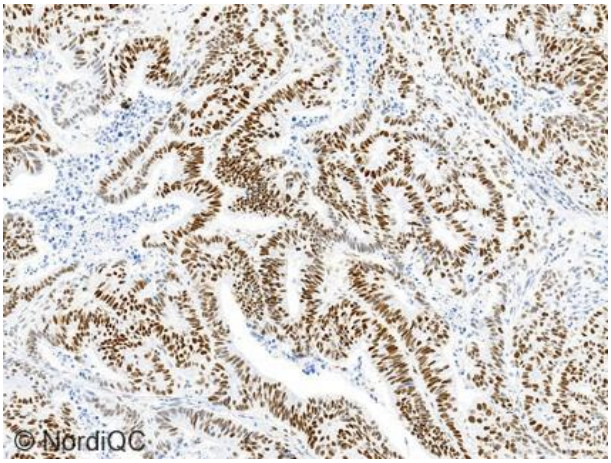


Fig. 2b
Insufficient staining reaction for MSH2 of the colon adenocarcinoma, tissue core no. 3, using same protocol as in Fig. 1b - same field as in Fig. 2a. The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result expected and shown in Fig. 2a. Especially note that the stromal cells are virtually negative. Also compare with Fig. 3b and 4b, same protocol.

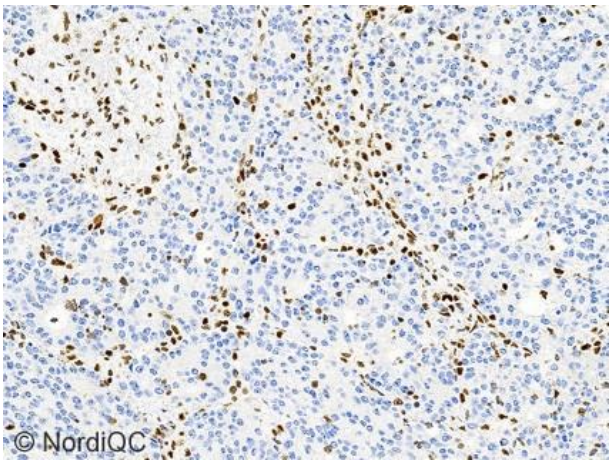


Fig. 3a
Optimal MSH2 staining of the colon adenocarcinoma, tissue core no. 4, with loss of MSH2 expression using same protocol as in Figs. 1a and 2a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

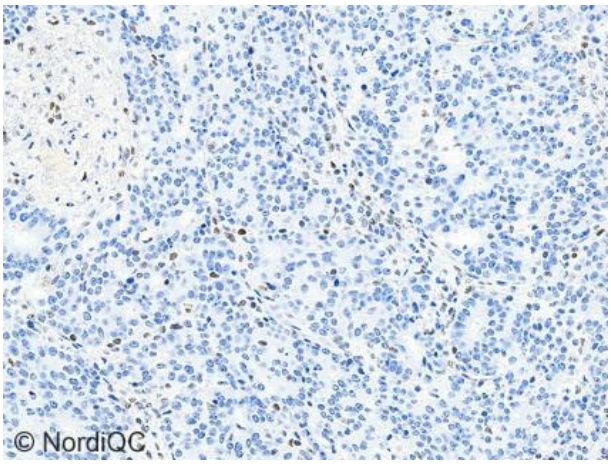


Fig. 3b
Insufficient MSH2 staining of the colon adenocarcinoma, tissue core no. 4, with loss of MSH2 expression using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. No staining reaction in the neoplastic cells is seen, but as also virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern cannot reliably be interpreted.

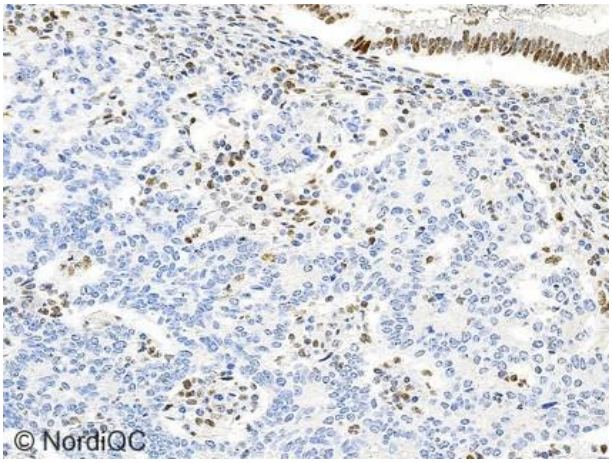


Fig.4a
Optimal MSH2 staining of the colon adenocarcinoma, tissue core no. 5, with loss of MSH2 expression using same protocol as in Figs. 1a and 2a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

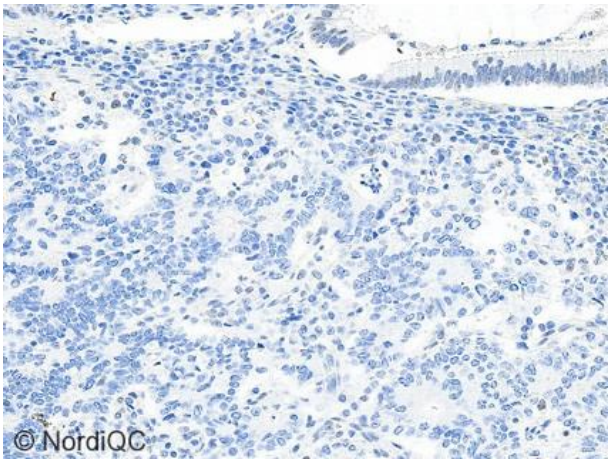


Fig. 4b
Insufficient MSH2 staining of the colon adenocarcinoma, tissue core no. 5, with loss of MSH2 expression using same protocol as in Figs. 1b and 2b – same field as in Fig. 3a. No staining reaction in the neoplastic cells is seen, but as no nuclear staining reaction in the normal stromal cells is present, the staining pattern cannot reliably be interpreted.

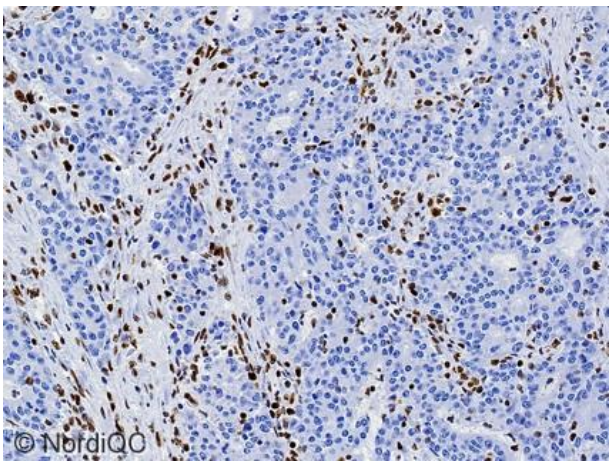


Fig.5a
Optimal MSH2 staining of the colon adenocarcinoma, tissue core no. 4, with loss of MSH2 expression using the mAb clone FE11, optimally calibrated, HIER in an alkaline buffer and a 3-step polymer based detection system on the Dako Autostainer. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. Compare with Fig. 5b.

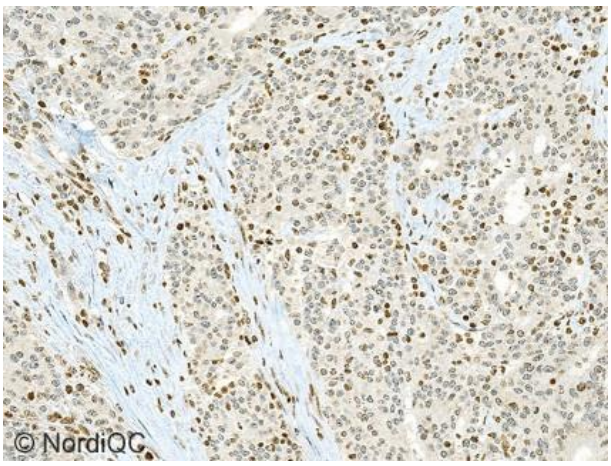


Fig. 5b
Insufficient staining reaction for MSH2 of the colon adenocarcinoma, tissue core no. 4, with loss of MSH2 expression using the mAb clone FE11, HIER in an alkaline buffer and a 3-step multimer based detection system on the Ventana BenchMark Ultra. The combination of a general cytoplasmic staining reaction and a weak false positive nuclear staining reaction in many the neoplastic cells complicates the interpretation. The mAb clone FE11 seems less successful on the Ventana BenchMark platform. Compare with Fig. 5a (same field).

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