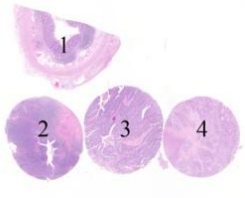
Assessment Run 41 2014

Mismatch Repair Protein MSH2 (MSH2)

Material

The slide to be stained for MSH2 comprised:



1. Appendix, 2. Tonsil, 3. Colon adenocarcinoma with normal MSH2 expression, 4. 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 adenocarcinoma no. 4, 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 41	155
Number of laboratories returning slides	143 (92%)

Results

143 laboratories participated in this assessment. Of these, 96 (67%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (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)

Performance history

This was the 2nd NordiQC assessment of MSH2. A slight decrease in the pass rate was seen compared to run 22, 2008. However, the number of participants has increased significantly and most laboratries participated in the MSH2 test for the first time.

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

	Run 22 2008	**Run 41 2014**
Participants, n=	51	143
Sufficient results	73%	67%

Conclusion

The mAb clones **FE11** and **G219-1129** could both be used to obtain optimal staining results for MSH2. Irrespective of the clone applied, HIER in an alkaline buffer and 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 general IHC system applied. The Ready-To-Use systems based on these two clones from Dako and Ventana, respectively, provided the highest proportion of sufficient and optimal results.

Tonsil is a recommendable 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.

Table 1. **Antibodies and assessment marks for MSH2, run 41**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **25D12**	12 1 1	Leica/Novocastra Diagnostic BioSystems Thermo/NeoMarkers	0	0	12	2	-	-
mAb clone **FE11**	10 6 6	Biocare Dako Millipore/Calbiochem	3	10	9	0	59%	80%
mAb clone **G219-1129**	11 8 1	BD Biosciences Cell Marque Monosan	4	6	6	4	50%	90%
mAb clone **GB12**	1	Millipore/Calbiochem	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **25D12 PA0048**	3	Leica/Novocastra	0	0	3	0	-	-
mAb clone **FE11 IR085**	23	Dako	20	2	1	0	96%	100%
mAb clone **FE11 PM219**	2	Biocare	0	2	0	0	-	-
mAb clone **FE11 MSG031**	1	Zytomed	1	0	0	0	-	-
mAb clone **G219-1129 760-4265**	50	Ventana/Cell Marque	26	19	3	2	90%	93%
mAb clone **G219-1129 286M-18**	5	Cell Marque	2	1	2	0	60%	-
mAb clone **G219-1129 MAD-000371QD**	2	Master Diagnostica	0	0	2	0	-	-
Total	143		56	40	39	8	-	
Proportion			39%	28%	27%	6%	67%	

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 41

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 Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/6)* or Bond Epitope Retrieval Solution 2 (BERS2; Leica) (1/6) as retrieval buffer. The mAb was diluted in the range of 1:10-1:150 depending on the total sensitivity of the protocol employed.
Using these protocol settings 8 of 10 (80%) 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) (4/13) as retrieval buffer. The mAb was diluted in the range of 1:50-1:800 depending on the total sensitivity of the protocol employed.
Using these protocol settings 9 of 10 (90%) laboratories produced an optimal staining result.

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

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	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**	2/6** (33%)	0/1	0/3	-	1/5 (20%)	-
mAb clone **G219-1129**	0/2	2/2	4/10 (40%)	-	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) or TRS pH 9 (efficient heating time 10-20 min. at 95-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (K8000/K8002) as detection system.
Using these protocol settings 22 of 22 (100%) laboratories produced a sufficient staining result.

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 (efficient heating time 16-64 min.) 8-48 min. incubation of the primary Ab and UltraView (760-500) +/- amplification kit or OptiView (760-700) +/- amplification kit as detection systems.
Using these protocol settings 43 of 46 (93%) laboratories produced a sufficient staining result.

Comments
In this assessment, the prevalent features of insufficient staining results were either characterized by a generally too weak staining reaction of the cells expected to be demonstrated or a poor signal-to-noise ratio complicating the interpretation. Too weak or false negative staining reaction was seen in 72% of the insufficient results (34 of 47). 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.
In the remaining insufficient results (28%) a poor signal-to-noise ratio was seen complicating the interpretation.

The mAb clones FE11 and G219-1129 could both be used to obtain an optimal staining result. It was observed that two Ready-To-use (RTU) systems based on these two clones (from Dako and Ventana), 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 typically adjusting incubation time of the primary Ab, efficient HIER time and/or choice of the detection system.
For the concentrated formats of the mAb clones FE11 and G219-1129, optimal results could only be obtained by HIER in an alkaline buffer (pH 8-9) together with a 3-step polymer/multimer based detection system as EnVision Flex+ (Dako), OptiView (Ventana) and Refine (Leica).

Occassionally, the mAb clone G219-1129 gave an aberrant cytoplasmic staining reaction in endothelial cells. This was seen both as a concentrated format and as RTU from different vendors. No single protocol parameter, as concentration of the primary Ab, HIER conditions, detection system etc., could be identified as the source. Most likely contamination with another Ab caused this aberrant staining pattern. A weak aberrant cytoplasmic staining reaction of endothelial cells was accepted provided that otherwise optimal results for MSH2 were seen.

In this assessment the mAb clone 25D12 showed an inferior performance both as concentrate and RTU format, as all 17 protocols based on this clone gave insufficient results. This clone produced a generally too weak nuclear staining for MSH2 and a simultaneous aberrant granular cytoplasmic staining reaction of the neoplastic cells in the colon adenocarcinoma with loss of MSH2, complicating the interpretation. The protocol settings applied for the mAb clone 25D12 were similar to the laboratories producing optimal

staining results with the mAb clones FE11 and G219-1129. Neither laboratory validated protocols nor protocols based on the guidelines form the vendors gave a sufficient result for the mAb clone 25D12.

Controls
Tonsil was found to be a recommendable positive tissue control for MSH2. 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. No nuclear staining reaction should be seen in the neoplastic cells, whereas a nuclear staining reaction must be seen in stromal cells.

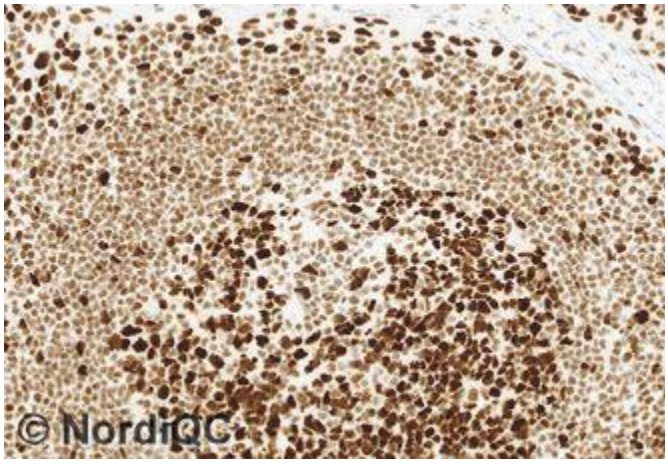


Fig. 1a (X200)
Optimal MSH2 staining of the tonsil using the mAb clone G219-1129, optimally calibrated, HIER in an alkaline buffer and a 3-step multimer based detection system. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Also compare with Figs. 2a and 3a, same protocol.

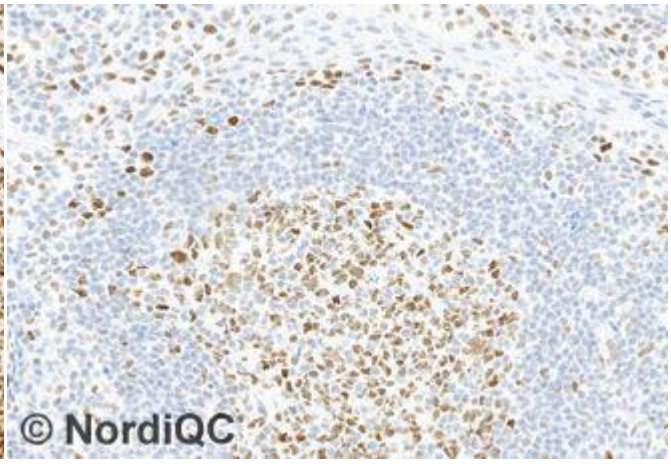


Fig. 1b (X200)
Insufficient MSH2 staining of the tonsil using the mAb clone G219-1129 with a protocol providing a too low sensitivity (2-step multimer based detection system and/or a too low concentration of the primary Ab) - same field as in Fig. 1a. Only the germinal centre B-cells are demonstrated, while mantle zone B-cells expressing low level MSH2 virtually are unstained. Also compare with Figs. 2b and 3b, same protocol.

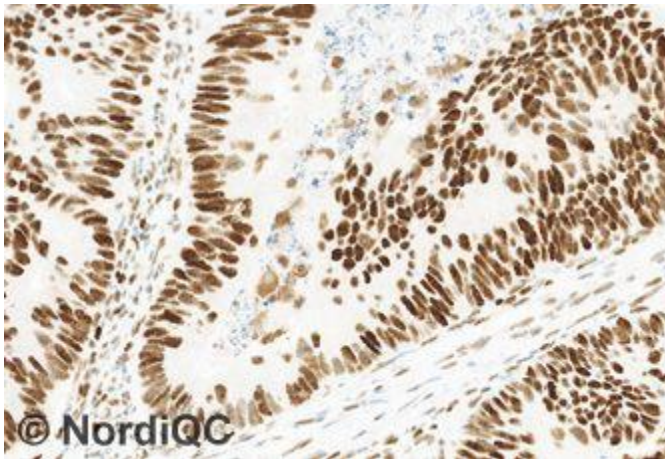


Fig. 2a (X200)
Optimal MSH2 staining of the colon adenocarcinoma tissue core no. 3 with normal MSH2 expression using same protocol as in Fig. 1a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained. No background staining is seen and a distinct nuclear staining reaction in the stromal cells is seen.

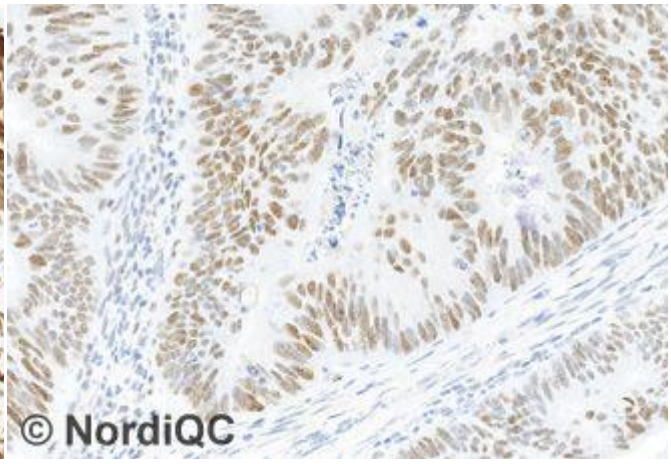


Fig. 2b (X200)
Insufficient staining reaction for MSH2 of the colon adenocarcinoma tissue core no. 3 using same protocol as in Fig. 1b - same field as in Fig. 2a. The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result expected and shown in Fig. 2. Especially note that the stromal cells are virtually negative. Also compare with Fig. 3b, same protocol.

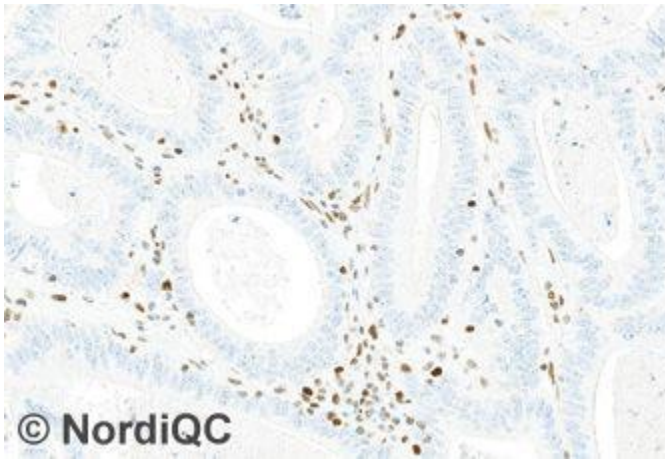


Fig. 3a (X200)
Optimal MSH2 staining of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Figs. 1a & 2a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

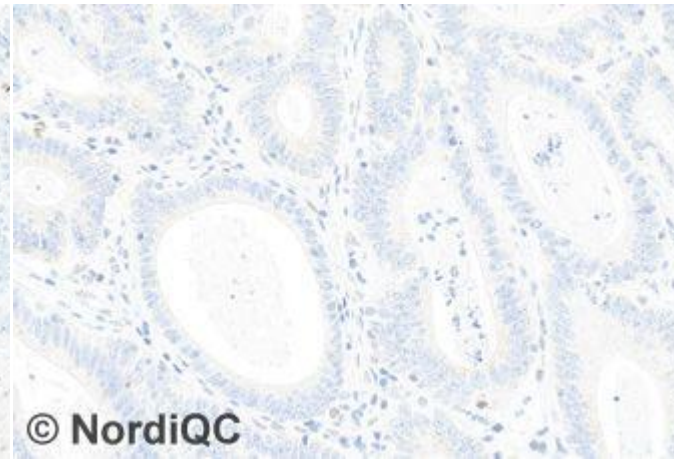


Fig. 3b (2400)
Insufficient MSH2 staining of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. No staining reaction in the neoplastic cells is seen, but as no nuclear staining reaction in the normal stromal cells is present, the staining pattern cannot reliably be interpreted.

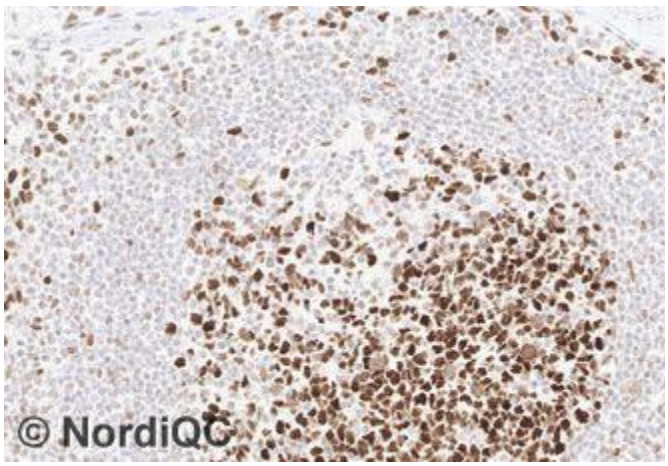


Fig.4a
Insufficient staining reaction for MSH2 using the mAb clone 25D12 with HIER in an alkaline buffer and a 3-step polymer based detection system. Mantle zone B-cells only show a faint or equivocal nuclear staining reaction, whereas germinal centre B-cells show a strong nuclear staining reaction. Also compare with Fig. 4b, same protocol.

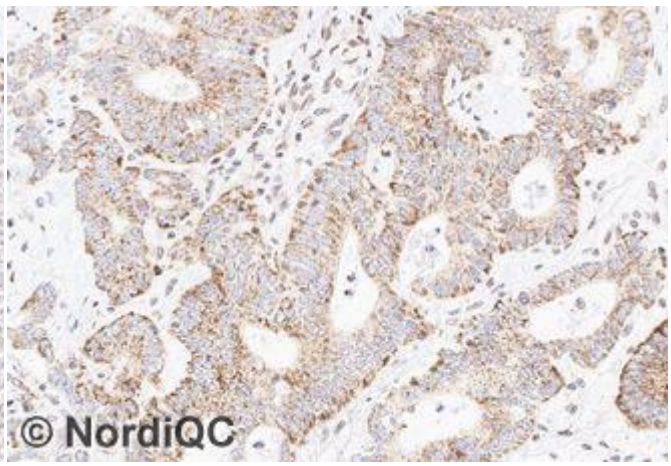


Fig. 4b
Insufficient staining reaction for MSH2 of the colon adenocarcinoma no. 4 with loss of MSH2 expression using same protocol as in Fig. 4a. The combination of an excessive granular cytoplasmic staining reaction in the neoplastic cells and a faint nuclear staining reaction of the stromal cells complicates the interpretation.
In this run all protocols (n=17) based on the mAb clone 25D12 gave an insufficient result.

SN/RR/MV/LE 19-06-2014