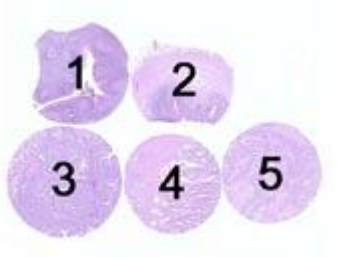
Assessment Run 32 2011

Mismatch repair protein MSH6 (MSH6)

Material

Mismatch Repair Protein MSH6



The slide to be stained for MSH6 comprised:

1. Tonsil, 2. Appendix, 3 – 5. Colon adenocarcinomas.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a MSH6 staining as optimal included:

- An at least weak to moderate nuclear staining reaction of almost all cells in the appendix.
- An at least weak to moderate nuclear staining reaction in the mantle zone B-cells and a moderate to strong nuclear staining reaction in the germinal centre B-cells in the tonsil.
- A moderate to strong nuclear staining in the majority of the neoplastic cells of the colon adenocarcinoma no. 3.
- A negative staining reaction in the neoplastic cells of the colon adenocarcinomas no. 4 & 5 and a distinct nuclear reaction in all other cells (a weak nuclear staining reaction in scattered neoplastic cells was accepted).
- A weak cytoplasmic reaction was accepted.

90 laboratories participated in this assessment. 33 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for MSH-6, run 32**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **44**	36 9 2 1 1 1 1	BD Biosciences Biocare Zytomed Abcam Cell Marque Master Diagnostica Zeta Corporation	0	10	33	8	20 %	-
mAb clone **PU29**	11	Leica/Novocastra	0	3	5	3	27 %	-
rmAb clone **EPR3945**	6	Epitomics	4	2	0	0	100 %	100 %
rmAb clone **EP49**	4	Epitomics	3	1	0	0	-	-
Ready-To-Use Abs								
mAb clone **44** **790-4455**	8	Ventana	0	1	5	2	12 %	-
mAb clone **44** **287M-17/18**	3	Cell Marque	0	3	0	0	-	-
mAb clone **44** **08-1374**	2	Zymed/Invitrogen	0	0	1	1	-	-
mAb clone **44 PM265**	2	Biocare	1	1	0	0	-	-
mAb clone **44** **0117-01**	1	Master Diagnostica	0	0	1	0	-	-
mAb clone **PU29** **PA0597**	1	Leica/Novocastra	0	1	0	0	-	-
mAb clone **2D4B5** **ZM-0387**	1	Zhongshan jinqiao	0	0	0	1	-	-
Total	90		8	22	45	15	-	

Proportion			9 %	24 %	50 %	17 %	33 %	

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

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
rmAb clone **EPR3945**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Cell Conditioning 1 (CC1) (BenchMark, Ventana) (2/3)* or Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:50-1:250 depending on the total sensitivity of the protocol employed. Using these protocol settings 5 out of 5 (100 %) laboratories produced a sufficient staining (optimal or good).
*(number of optimal results/number of laboratories using this buffer)

rmAb clone **EP49**: The protocols giving an optimal result were all based on HIER using either CC1 (BenchMark, Ventana) (1/2), TRS pH 9 (3-in-1) (Dako) (1/1) or TRS pH 9 (Dako) (1/1) as the retrieval buffer. The mAb was typically diluted in the range of 1:100-1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 out of 4 (100 %) laboratories produced a sufficient staining (optimal or good).

Ready-To-Use Abs
mAb clone **44** (prod. no. PM265, Biocare): The protocol giving an optimal result was based on HIER in a Pressure Cooker using Reveal Decloaker pH 6 (Biocare) and an incubation time of 30 min in the primary Ab and MACH4 (4U534, Biocare) as the detection system.

The most frequent causes of insufficient stains were:

- Less successful primary Ab
- Too low concentration of the primary Ab
- Insufficient HIER (use of a non-alkaline buffer and/or too short efficient HIER)
- Use of low sensitive detection systems.

In this assessment the prevalent feature of an insufficient staining was a too weak or false negative nuclear staining reaction of the majority of the cells expected to be demonstrated. The majority of the laboratories were able to demonstrate MSH6 in the cells with a high antigen expression as the proliferating germinal centre B-cells and the basal epithelial cells of the appendix, whereas the demonstration of MSH6 in cells with a low antigen expression as the resting mantle zone B-cells, smooth muscle cells and stromal cells could only be obtained by an optimally calibrated protocol. In this context it has to be emphasized, that the identification of loss of MSH6 in tumours is characterized by a negative staining reaction of the neoplastic cells, wherefore it is of decisive importance that the normal cells within and around the neoplastic cells show a distinct positive nuclear staining reaction, serving as internal positive control. A too weak or completely false negative staining was seen in 78 % of the insufficient results (47 out of 60) whereas in 22 % of the insufficient results both a too weak staining and an excessive background staining was seen.
The most widely used Ab was the mAb clone 44 either as a concentrate or as a Ready-To-Use product being used by 66 out of the 90 participating laboratories. The proportion of sufficient results based on this clone was very low as only 16 out of the 66 laboratories obtained a sufficient mark (24 %) and only one of these was assessed as optimal (2 %). The mAb clone 44 was found to be very challenging to provide a robust and easy interpretable staining result as the clone seemed to have a relative low affinity for the specific nuclear MSH6 antigen combined with a cross-reaction with a cytoplasmic protein. If the laboratories used a reduced titre of the clone in order to eliminate the unspecific cytoplasmic staining reaction, the specific nuclear staining was significantly reduced giving a too weak reaction, and if the mAb clone 44 was applied within a highly sensitive IHC system e.g. based on HIER in an alkaline buffer and a 3-step labelled polymer system, an excessive background and cytoplasmic staining reaction was seen compromising the interpretation.
The optimal result for MSH6 based on the mAb clone 44 was obtained by use of a Ready-To-Use system from Biocare using HIER at pH 6 and a highly sensitive 3-step labelled polymer detection system, MACH-4. The combination of usage of a diluent with background reducing capability and a very sensitive detection system might be the explanation for the successful application of the mAb clone 44. 2 out of 2 laboratories using the Ready-To-Use format of the mAb clone 44 from Biocare obtained a sufficient result.
The newly launched rmAbs clone EPR3945 and EP49 were found to be the most robust markers for MSH6, as all 10 out of 10 protocols based on one of these clones gave a sufficient result and an optimal staining result could be obtained by the use on both an open IHC platform as the Dako Autostainer Link and on a fully automated IHC platform as the Ventana BenchMark Ultra. The staining result obtained by the rmAb clones EPR3945 and EP49 was characterized by a very high signal-to-noise ratio as a strong and distinct nuclear staining was seen while virtually no interfering cytoplasmic staining was seen. The optimal results were all based on HIER in an alkaline

buffer and typically with a 3-step polymer/multimer based detection system. The rmAb clone EPR3945 is classified as a Research-Use-Only product and the clone EP49 as an In-Vitro-Diagnostic product, both Epitomics. Tonsil was found to be a recommendable positive control for MSH6: The mantle zone B-cells must show at least a weak to moderate nuclear staining, while a moderate to strong staining must be seen in the proliferating germinal centre B-cells.

Conclusion

In this assessment the rmAb clones EPR3945 and EP49 were the most successful and robust Abs for MSH6, while clones 44 and PU29 gave relatively insufficient results. HIER in an alkaline buffer and the use of a 3-step polymer/multimer based detection system gave the most robust protocol. Tonsil is a recommendable positive control for MSH6 in which virtually all the mantle zone B-cells must show at least a weak to moderate nuclear staining, while a moderate to strong staining must be seen in the proliferating germinal centre B-cells.

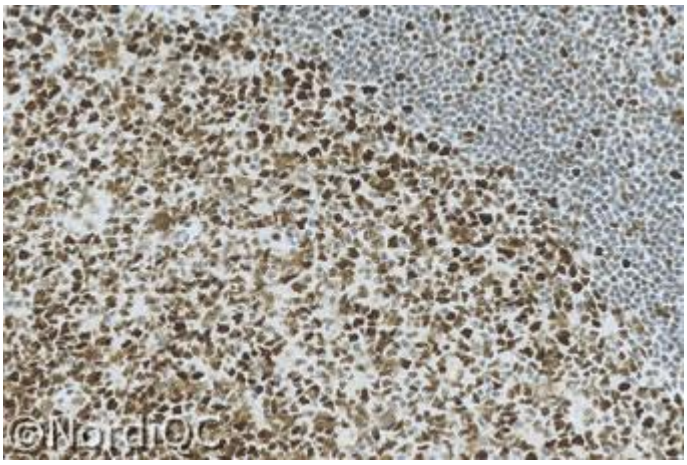


Fig. 1a
Optimal staining for MSH6 of the tonsil using the rmAb clone EP49 optimally calibrated, HIER in an alkaline buffer and a 3-step polymer based detection system. Virtually all the mantle zone B-cells show a distinct, moderate to strong nuclear staining, while the germinal centre B-cells show a strong nuclear staining.

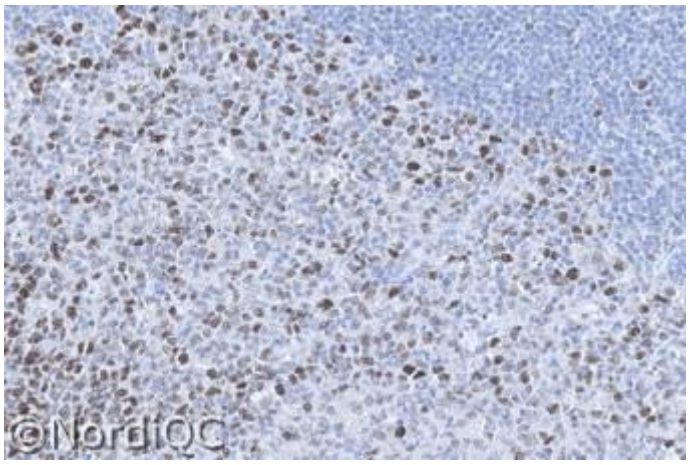


Fig. 1b
Insufficient staining for MSH6 of the tonsil using the mAb clone 44. by a protocol with a too low sensitivity (2-step polymer and too low. conc. of the primary Ab), same field as in Fig. 1a.
Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing limited MSH6 are virtually unstained.
Also compare with Figs. 2b. & 3b., same protocol.

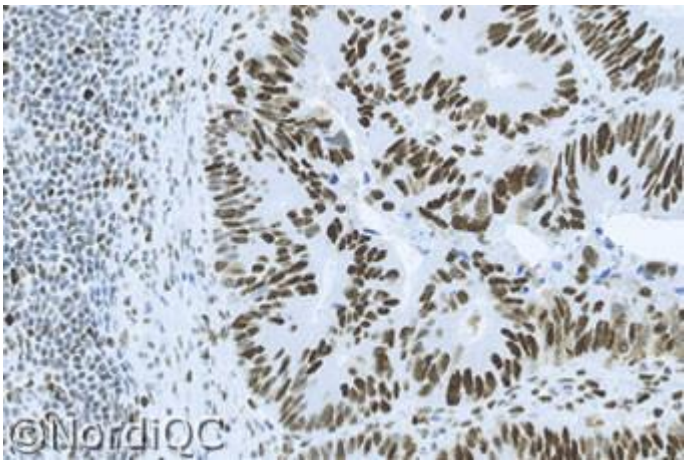


Fig. 2a
Optimal staining for MSH6 of the colon adenocarcinoma no. 3 with intact MSH6 protein using same protocol as in Fig. 1a.
The majority of the epithelial and the stromal cells show a moderate to strong nuclear staining. No background staining is seen.

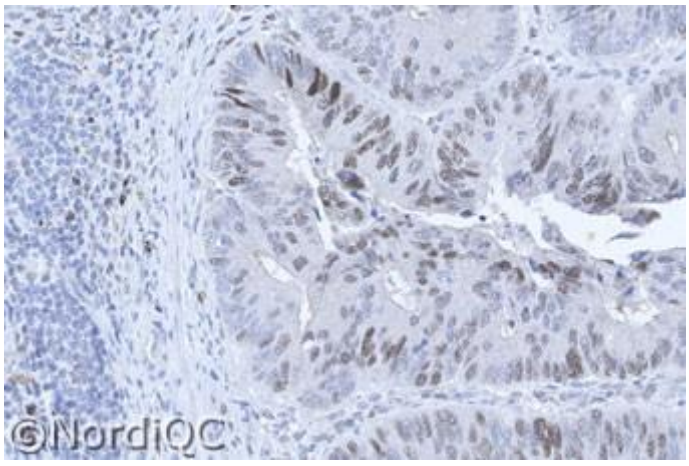


Fig. 2b
Insufficient staining for MSH6 of the colon adenocarcinoma no. 3 with intact MSH6 protein using same protocol as in Fig. 1b., same field as in Fig. 2a.
The proportion of positive cells and the intensity of the staining reaction is significantly reduced compared to the result in Fig. 2a.
Also compare with Fig. 3b., same protocol.

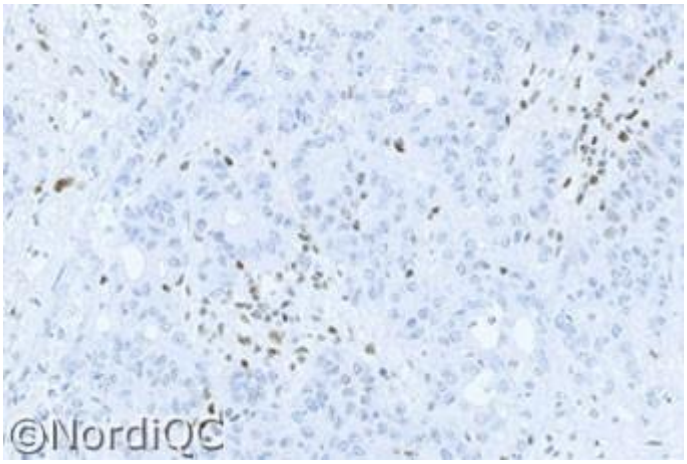


Fig. 3a
Optimal staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Figs. 1a. & 2a.
The neoplastic cells are negative, while the remnants of entrapped lymphocytes and stromal cells show a distinct nuclear staining, serving as internal positive control.

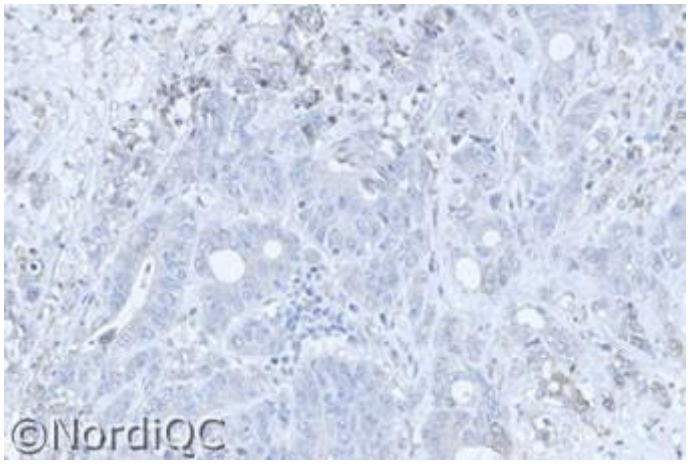


Fig. 3b
Insufficient staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Figs. 1b. & 2b., same field as in Fig. 3a.
No nuclear staining reaction is seen in the neoplastic cells, but as virtually no nuclear staining reaction is seen in the normal cells as stromal cells, the staining pattern can not reliably be interpreted. Also note the weak cytoplasmic staining complicating the interpretation.

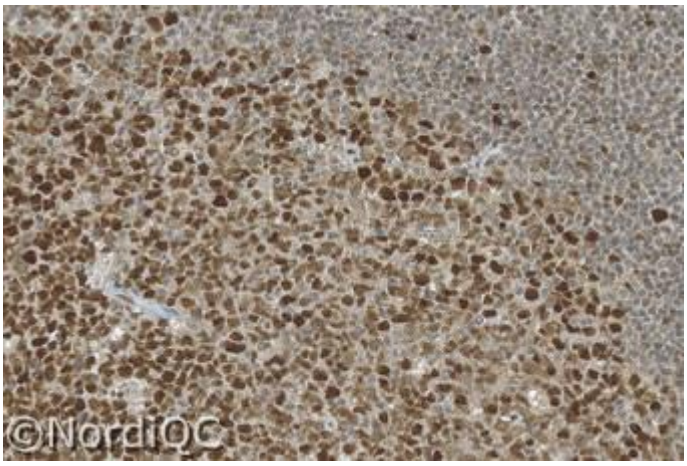


Fig. 4a
Staining for MSH6 of the tonsil using the mAb clone 44 by HIER in an alkaline buffer and a 3-step polymer based detection system – same field as in Fig. 1a. Virtually all the mantle zone B-cells show a moderate to strong nuclear staining, while the germinal centre B-cells show a strong nuclear staining.
However also compare with Fig. 4b, same protocol.

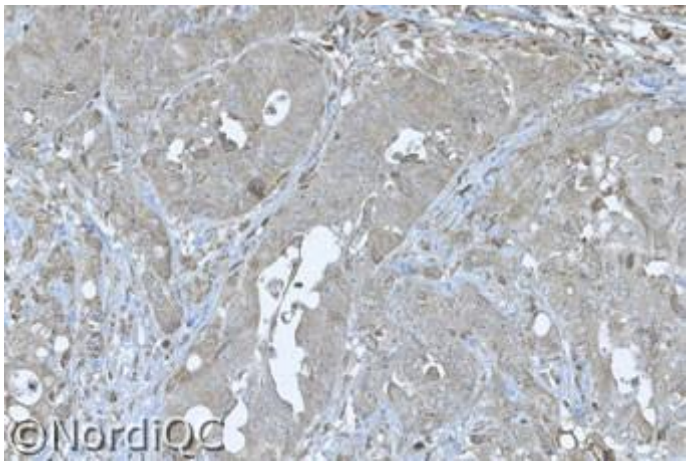


Fig. 4b
Insufficient staining for MSH6 of the colon adenocarcinoma no. 5 with loss of MSH6 protein using same protocol as in Fig. 4a.
The excessive cytoplasmic staining in both the neoplastic cells and in the stromal cells obscures the interpretation of the nuclear staining.
This staining pattern was typically seen when the mAb clone 44 was applied with a high sensitive protocol.

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