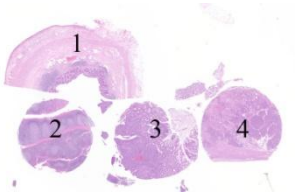
Assessment Run 41 2014

Mismatch Repair Protein PMS2 (PMS2)

Material
The slide to be stained for PMS2 comprised:



1. Appendix, 2. Tonsil, 3. Colon adenocarcinoma with normal PMS2 expression, 4. Colon adenocarcinoma with loss of PMS2 expression.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a PMS2 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 of virtually all neoplastic cells in the colon adenocarcinoma no. 3
- No nuclear staining reaction of the neoplastic cells in the colon adenocarcinomas no. 4, but a distinct nuclear staining reaction in the vast majority of other cells (stromal cells, lymphocytes etc).

A general weak cytoplasmic staining reaction was accepted.

Participation

Number of laboratories registered for PMS2, run 41	144
Number of laboratories returning slides	131 (91%)

Results
131 laboratories participated in this assessment. Of these, 85% (n=111) 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 were:

- Use of less sensitive detection systems
- Too low concentration of the primary antibody
- Insufficient heat induced epitope retrieval.

Performance history
This was the 1st NordiQC assessment of PMS2. Slightly higher proportion of sufficient results was seen compared to NordiQC runs of the other mismatch repair protein markers.

Conclusion
The mAb clones **A16-4** and **M0RG4** and the rmAb clones **EP51** and **EPR3947** could all be used to obtain an optimal staining result for PMS2.The rmAb clones **EP51** and **EPR3947** provided optimal staining results on the three main platforms from Dako, Leica and Ventana.
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 must be carefully calibrated.
Tonsil is recommendable as positive tissue control for PMS2. Mantle zone B-cells must show an at least weak to moderate, distinct nuclear staining reaction, while moderate to strong, distinct nuclear staining reaction must be seen in proliferating germinal centre B-cells. Tumour tissue, e.g. colon adenocarcinoma with loss of PMS2 expression must be used as negative tissue control, in which no nuclear staining reaction of the neoplastic cells must be seen.

Table 1. **Antibodies and assessment marks for PMS2, run 41**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A16-4**	19 2	BD Biosciences Biocare	9	8	2	2	81%	100%
mAb clone **M0R4G**	4	Leica/Novocastra	1	2	1	0	-	-
mAb clone **MRQ-28**	2 1 1	Cell Marque DCS Monosan	0	2	0	2	-	-
rmAb clone **EPR3947**	3	Epitomics	2	1	0	0	-	-
rmAb clone **EP51**	13 4	Dako Epitomics	10	7	0	0	100%	100%
Ready-To-Use antibodies								
mAb clone **A16-4 PM344AA**	3	Biocare	3	0	0	0	-	-
mAb clone **MRQ-28 288M-18**	1	Cell Marque	0	0	1	0	-	-
mAb clone **MRQ-28 MAD-000070QD**	1	Master Diagnostica	1	0	0	0	-	-
rmAb clone **EPR3947 760-4531**	47	Ventana/Cell Marque	19	17	8	3	77%	89%
rmAb clone **EPR3947 288R-18**	5	Cell Marque	2	3	0	0	100%	-
mAb clone **EP51 IR087**	25	Dako	15	9	1	0	96%	100%
Total	131		62	49	13	7	-	
Proportion			47%	38%	10%	5%	85%	

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

Detailed analysis of PMS2, Run 41

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **A16-4**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (2/3)*, TRS pH 9 (Dako) (3/4) or Bond Epitope Retrieval Solution 2 (BERS2; Leica) (4/7) as retrieval buffer. The mAb was typically diluted in the range of 1:75-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings 13 of 13 (100%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **M0R4G**: One protocol with an optimal result was based on HIER using BERS1 (Leica) (1/1) as retrieval buffer. The dilution factor was 1:100 using a 3-step polymer based detection system (RE7280-K, Leica).

rmAb clone **EPR3947**: Protocols with optimal results were all based on HIER using Cell Conditioning 1 (CC1; Ventana) (2/3) as retrieval buffer. The rmAb was diluted in the range of 1:75-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings 2 of 2 (100%) laboratories produced an optimal staining result.

rmAb clone **EP51**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako) (2/4), TRS pH 9 (Dako) (3/3), CC1 (Ventana) (2/4), BERS2 (Leica) (2/2) or Tris-EDTA/EGTA pH 9 (1/3) as retrieval buffer. The rmAb was typically diluted in the range of 1:20-1:50 depending on the total sensitivity of the protocol employed. Using these protocol settings 14 of 14 (100%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for PMS2 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 **A16-4**	4/6** (67%)	-	0/3	-	3/5 (60%)	-
rmAb clone **E51**	5/7 (71%)	-	2/4	-	2/2	-

* 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 **A16-4**, product no. **PM344AA**, Biocare, IntelliPath:
One protocol with an optimal result was based on HIER using Borg Decloaker pH 9.5 in a pressure cooker and MACH4 (M4U534) as detection system.

rmAb clone **EPR3947,** product no. **760-4531**, Ventana/Cell Marque, BenchMark XT/Ultra:
Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (efficient heating time 32-92 min.), 16-120 min. incubation of the primary Ab. and OptiView (760-700) +/- amplification kit as detection system. Using these protocol settings 25 of 28 (89%) laboratories produced a sufficient staining result.

rmAb clone **EP51**, product no. **IR087**, 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.

Comments
In this first NordiQC assessment of PMS2, the prevalent feature of an insufficient staining was a too weak or completely false negative staining reaction in cells expected to be demonstrated. Too weak or false negative staining reaction was seen in 80% of the insufficient results (16 of 20). The majority of laboratories were able to demonstrate PMS2 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 with normal PMS2 expression. Demonstration of PMS2 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. In this context it has to be emphasized, that identification of loss of PMS2 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 (20%) poor signal-to-noise ratio or impaired morphology was seen which complicated correct interpretation.
Using a concentrated Ab, optimal result could be obtained by many clones as listed in table 1. Irrespective of the clone applied, careful calibration of the titre and efficient HIER in an alkaline buffer in combination with a sensitive non-biotin based detection system were the main protocol prerequisites for optimal results. Especially use of 3-step polymer/multimer based detection systems provided higher proportions of optimal results. Protocols based on a 3-step system as EnVision FLEX+ (Dako), OptiView (Ventana) or Refine (Leica) gave a proportion of 50% optimal results (14 of 28), whereas 33% were assessed as optimal using a 2-step system as EnVision Flex (Dako) and UltraView (Ventana) (7 of 21).
The rmAb clone EP51 as a concentrated format provided a pass rate of 100%, and optimal results could be obtained on the three main IHC systems from Dako, Leica and Ventana. The mAb clone A16-4 also provided a high overall pass rate, however, no optimal results were generated on the Ventana system.

The Ready-To-Use (RTU) systems from Ventana and Dako based on the rmAb clones EPR3947 and EP51, respectively, gave a high proportion of sufficient and optimal results. Optimal results for the Dako RTU system could both be obtained by using the official protocol recommendations given by Dako but also by laboratory modified protocol settings (typically adjusting incubation time of the primary Ab and/or choice of detection system). The Ventana RTU system only provided optimal results using laboratory modified protocol settings (typically using OptiView +/- amplification as detection system and/or prolonged incubation time of the primary Ab), whereas no optimal results were obtained using the Ventana recommended protocol settings (based on 32 min. incubation time of the primary Ab and UltraView or iView with amplification as detection system).

Controls
Tonsil was found to be a recommendable positive tissue control for PMS2. Virtually all mantle zone B-cells must show at 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 PMS2 expression is recommended as negative tissue control for PMS2. No nuclear staining reaction should be seen in the neoplastic cells, whereas a nuclear staining reaction must be seen in stromal cells.

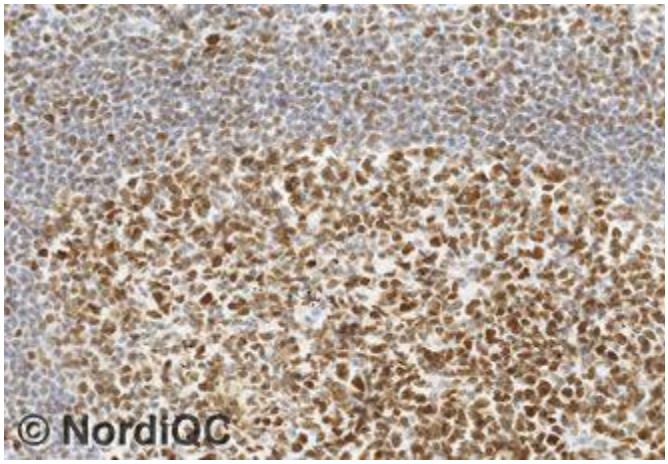


Fig. 1a (X200)
Optimal PMS2 staining of the tonsil using the rmAb clone EP51, HIER in an alkaline buffer and a 3-step polymer based detection system. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Also compare with Figs. 2a-4a, same protocol.

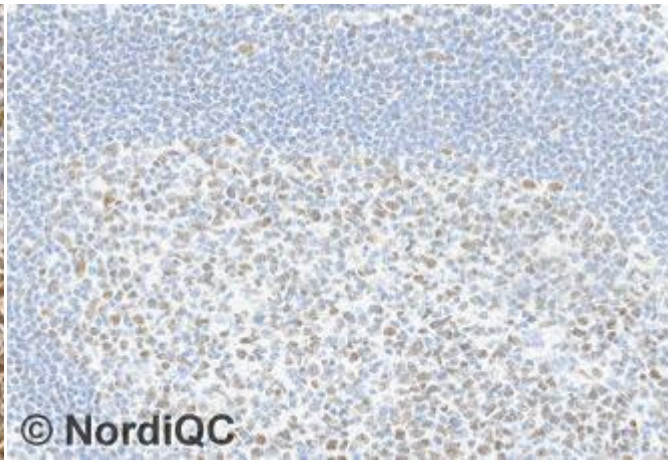


Fig. 1b (X200)
Insufficient PMS2 staining of the tonsil using the rmAb clone EP51 with a protocol providing a too low sensitivity (2-step multimer based detection system and/or a too low concentration of the primary Ab) - same field as in Fig. 1a. Only the germinal centre B-cells are demonstrated, while the mantle zone B-cells expressing low level PMS2 are virtually unstained. Also compare with Figs. 2b and 3b, same protocol.

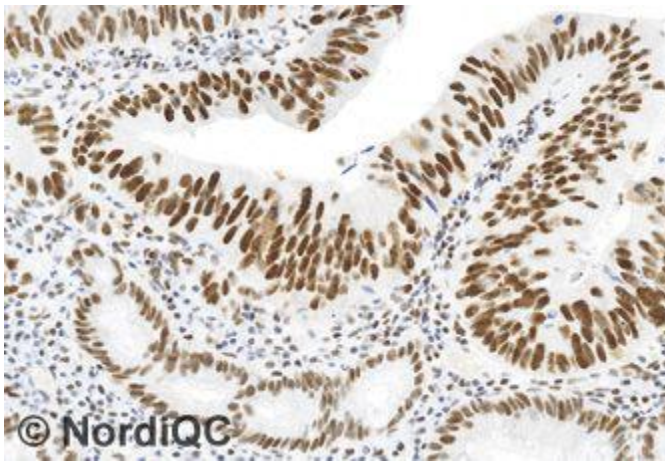


Fig. 2a (X200)
Optimal PMS2 staining of the colon adenocarcinoma tissue core no. 3 with normal PMS2 expression using same protocol as in Fig. 1a. Virtually all the neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained. No background staining is seen and a distinct nuclear staining reaction in the stromal cells is seen.

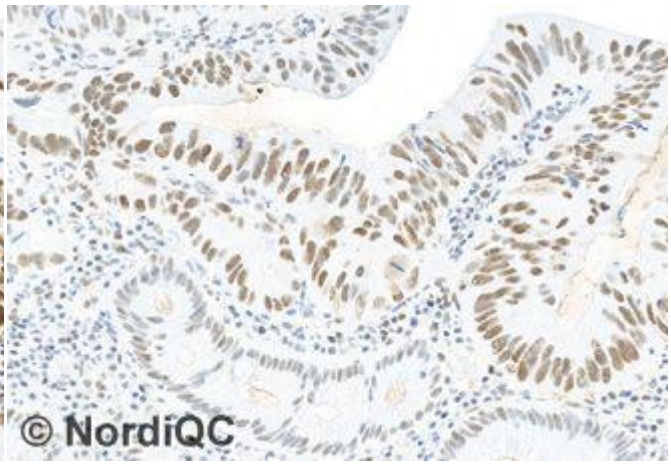


Fig. 2b (X200)
Insufficient PMS2 staining of the colon adenocarcinoma tissue core no. 3 using same protocol as in Fig. 1b - same field as in Fig. 2a.
The proportion of positive cells and the intensity of the staining reaction are significantly reduced compared to the result expected and shown in Fig. 2. Especially note that stromal cells are virtually negative and a stronger intensity is seen in neoplastic cells compared to the level seen in remnants of normal epithelial cells. Also compare with Fig. 3b, same protocol.

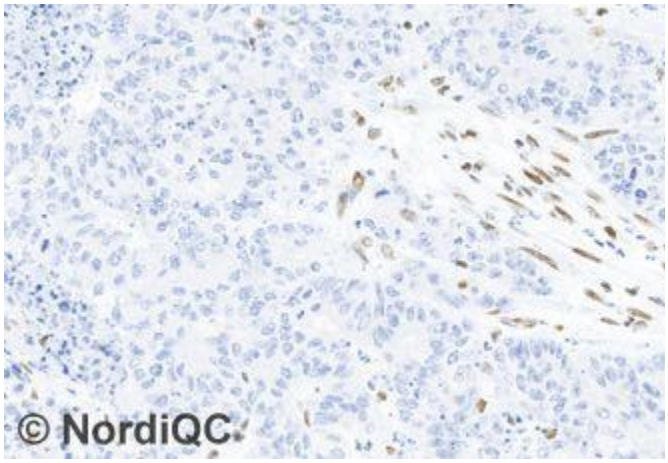


Fig. 3a (X200)
Optimal PMS2 staining of the colon adenocarcinoma no. 4 with loss of PMS2 expression using same protocol as in Figs. 1a & 2a. The neoplastic cells are negative, while lymphocytes and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

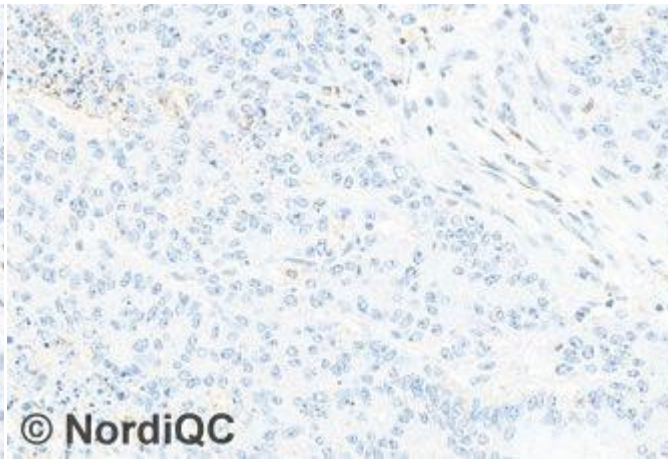


Fig. 3b (X200)
Insufficient PMS2 staining of the colon adenocarcinoma no. 4 with loss of PMS2 expression using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. No staining reaction in the neoplastic cells is seen, but as virtually no nuclear staining reaction is seen in the normal stromal cells, the staining pattern cannot reliably be interpreted.

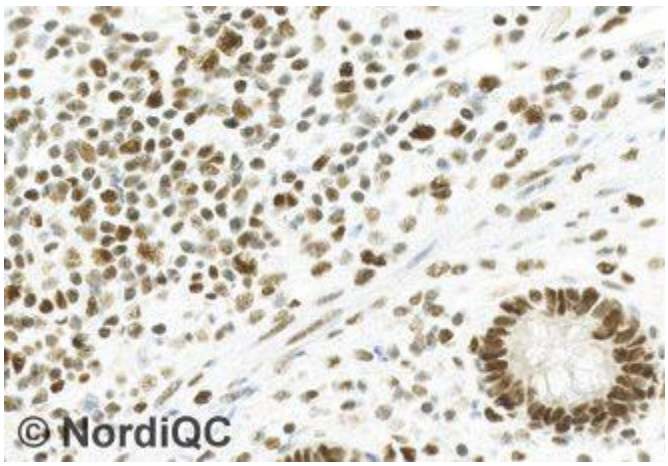


Fig.4a (x300)
Optimal PMS2 staining of the appendix using same protocol as in Figs. 1a - 3a. In both cells with a reduced PMS2 expression as stromal cells and lymphocytes and also in cells with a high level expression as epithelial cells a homogenous and uniformly nuclear staining reaction is seen. This pattern typically was seen in optimal results based on conventional 3-step polymer based detection kits as EnVision FLEX+ (Dako) and Quanto (Thermo).

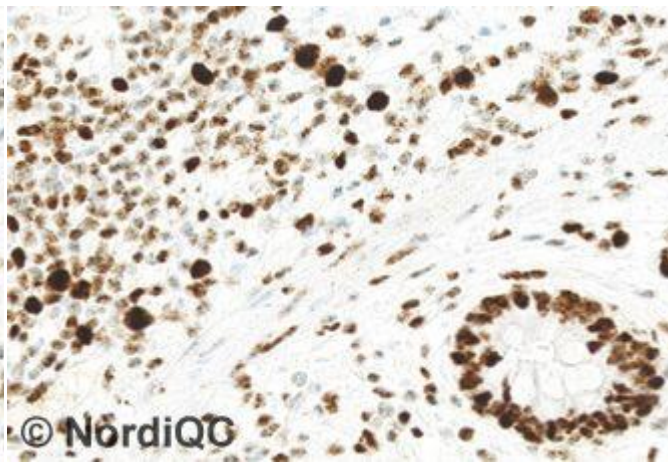


Fig. 4b (X300)
Optimal PMS2 staining of the appendix using a 3-step multimer based detection system in combination with tyramide signal amplification (OptiView+amplification, Ventana). The staining reaction and DAB precipitation is more granular, which especially is seen in cells with low level PMS2 expression. However the overall staining result was fully adequate and thus evaluated as optimal.

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