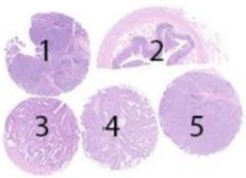
Assessment Run 53 2018

Mismatch repair protein PMS2 (PMS2)

Material
The slide to be stained for PMS2 comprised:



1. Tonsil 2. Appendix 3. Colon adenocarcinoma with normal PMS2 expression, 4-5. Colon adenocarcinomas with loss of PMS2 expression.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing PMS2 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 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 and 5, but a weak to moderate 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 53	253
Number of laboratories returning slides	246 (97%)

Results
246 laboratories participated in this assessment. 218 (89%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes 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 or too high concentration of the primary Ab
- Insufficient Heat Induced Epitope Retrieval (HIER)

Performance history
This was the second NordiQC assessment of PMS2. A small increase of the pass rate was seen compared to run 41, 2014.

Table 2. **Proportion of sufficient results for PMS2 in the two NordiQC runs performed**

	Run 41 2014	Run 53 2018
Participants, n=	131	246
Sufficient results	85%	89%

Conclusion
Optimal staining results could be obtained with the rabbit monoclonal Ab (rmAb) clones **EP51** and **EPR3947** and the mouse monoclonal Ab (mAb) clones **A16-4 and MRQ-28.** Only mAb clone **MOR4G** failed to produce optimal staining results, this clone was only used by three laboratories, however. Irrespective of the clone applied, efficient HIER in alkaline buffer, use of a sensitive polymer/multimer detection system and careful calibration of the primary Ab were the most important prerequisites for an optimal staining result. The concentrated (Conc) format of the rmAb clone **EP51** provided a high proportion of optimal staining results on all four main stainer platforms - Omnis (Dako), Autostainer (Dako), Bond (Leica) and BenchMark (Ventana).
In general, Ready-To-Use (RTU) systems performed slightly better than Conc Abs. The highest proportion of sufficient staining results was seen using the rmAb clone EP51 based Dako Autostainer RTU system and the rmAb clone EPR3947 based Ventana Benchmark RTU system. Using the recommended protocol settings, the Dako Autostainer RTU system was the most successful assay with an overall pass rate of 100% of which 80% were optimal.

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 a 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. Stromal cells within the tumour tissue must exhibit a distinct nuclear staining reaction serving as internal positive tissue control.

Table 1. **Antibodies and assessment marks for PMS2, run 53**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **A16-4**	9 3 2	BD Biosciences Zytomed Bicare	6	4	3	1	71%	75%
mAb clone **M0R4G**	3	Leica/Novocastra	0	2	0	1	-	-
mAb clone **MRQ-28**	2	Cell Marque	2	0	0	0	-	-
rmAb clone **EP51**	30 6 6 4 1 1 1 1	Dako/Agilent Epitomics Nordic Biosite Cell Marque Bio SB Biogenex Immunologic Leica	31	14	4	1	90%	91%
rmAb clone **EPR3947**	1	Epitomics	1	0	0	0	-	-
Ready-To-Use antibodies								
mAb clone **A16-4 PM344AA**	3	BioCare	3	0	0	0	**-**	**-**
mAb clone **A16-4 PDM171**	2	Diagnostic Biosystems	0	1	1	0	-	-
mAb clone **A16-4 790-5094**	4	Ventana/Roche	2	2	0	0	-	-
mAb clone **A16-4 MAD-000744QD**	1	Master Diagnostica	1	0	0	0	-	-
mAb clone **MRQ-28 288M-18**	4	Cell Marque	1	0	1	2	**-**	**-**
rmAb clone **EP51 IR087**	41	Dako/Agilent	26	14	1	0	98%	100%
rmAb clone **EP51 IR087** [3]	17	Dako/Agilent	13	4	0	0	100%	-
rmAb clone **EP51 IR087** [4]	2	Dako/Agilent	1	1	0	0	-	-
rmAb clone **EP51 RMA-0775**	1	Maixin	1	0	0	0	-	-
rmAb clone **EP51 MAD-000681QD**	1	Master Diagnostica	0	1	0	0	-	-
rmAb clone **EP51 8328-C010**	2	Sakura Finetek	2	0	0	0	-	-
rmAb clone **EPR3947 288R-18**	15	Cell Marque	8	4	2	1	80%	-
rmAb clone **EPR3947 760-4531**	83	Ventana/Roche	45	28	9	1	88%	89%
Total	246		145	75	21	7	-	
Proportion			58%	31%	8%	3%	89%	

1) Proportion of sufficient stains (optimal or good),
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer) but used by laboratories on the full-automatic Dako Omnis system.
4) RTU system developed for the Dako/Agilent semi-automatic system (Dako Autostainer) but used by laboratories on different platforms (e.g. Ventana Benchmark and manual).

Detailed analysis of PMS2, Run 53
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 Bond Epitope Retrieval Solution 2 (BERS2; Leica) (4/10)*, Tris-EDTA/EGTA pH 9 (1/1) or Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:100-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 9 of 12 (75%) laboratories produced an optimal staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **MRQ-28**: Protocols with optimal results were all based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) (1/1) or TRIS pH 9 (1/1) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:30 depending on the total sensitivity of the protocol employed. Using these protocol settings, 2 of 2 laboratories produced an optimal staining result.

rmAb clone **EP51**: Protocols with optimal results were all based on HIER using BERS2 (Leica) (10/12), Cell Conditioning Solution 1 (CC1, Ventana/Roche) (9/17), Target Retrieval Solution High pH (TRS High pH, Dako/Agilent) (7/8), TRS pH 9 (3-in-1) (Dako/Agilent) (4/6), or HIER Buffer M, pH 8 (Thermo Scientific) (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:10-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 40 of 44 (91%) laboratories produced a sufficient staining result.

rmAb clone **EPR3947**: One protocol with an optimal result was based on HIER using CC1 (Ventana/Roche) (1/1) as retrieval buffer. The primary Ab was diluted 1:300 and stained using a 3-step multimer based detection system (OptiView, Ventana/Roche) with tyramide signal amplification.

Table 3. **Proportion of optimal results for PMS2 for the most commonly used antibodies as concentrates 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 **A16-4**	-	-	-	-	-	-	4/10** (40%)	-
rmAb clone **EP51**	5/7 (71%)	-	6/7 (86%)	-	9/17 (53%)	-	10/12 (83%)	-

* 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.

mAb clone **A16-4**, product no. **790-5094**, Ventana/Roche, Ventana Benchmark GX/XT/Ultra:
Two protocols with optimal results used similar protocol settings based on HIER in CC1 (Ventana/Roche) (efficient heating time for 92 min. at 100°C), 32 or 44 min. incubation at 36°C of the primary Ab and OptiView (Ventana/Roche, 760-700) with tyramide signal amplification (760-099 / 860-099) as detection system.

mAb clone **A16-4**, product no. **MAD-000744QD**, Master Diagnostica, MD-Stainer:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 for 20 min., 40 min. incubation of the primary Ab and Master Polymer Plus (Master Diagnostica, MAD-000230QP) as detection system.

rmAb clone **EP51**, product no. **IR087**, 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 95-99°C), 20-40 min. incubation of the primary Ab and EnVision FLEX/FLEX+ (Dako/Agilent K8000/K8002) as detection system. Using these protocol settings, 40 of 40 (100%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **EP51**, product no. **RMA-0775**, Maixin, manual:
One protocol with an optimal result was based on HIER using Tris-EDTA/EGTA pH 9 (Waterbath) for 20 min., 60 min. incubation of the primary Ab and MaxVision III DAB (Maixin) as detection system.

rmAb clone **EP51,** product no. **8328-C010**, Sakura Finetek, Genie:
Two protocols with optimal results used identical protocol settings based on 45 min. HIER using Sakura Finetek Tissue-Tek Genie High pH Antigen Retrieval Buffer, 30 min. incubation of the primary Ab and Tissue-Tek PRO DAB Detection Kit (8826-K250) 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 CC1 (efficient heating time 32-92 min.), 20-80 min. incubation of the primary Ab. and OptiView (760-700) +/- tyramide signal amplification kit as detection system. Using these protocol settings, 58 of 65 (89%) laboratories produced a sufficient staining result (optimal or good).

Table 4 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 intended IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for PMS2 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS mAb clone **A16-4, 790-5094**	100% (2/2)	50% (1/2)	100% (2/2)	50% (1/2)
Dako AS rmAb clone **EP51, IR087**	100% (20/20)	80% (16/20)	95% (20/21)	48% (10/21)
VMS rmAb clone **EPR3947, 760-4531**	67% (2/3)	33% (1/3)	89% (71/80)	55% (44/80)

* 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 observations in the previous assessment of PMS2, run 41 2014, the prevalent feature of an insufficient staining reaction was a too weak or false negative nuclear staining reaction of the majority of cells expected to be demonstrated. Too weak or false negative staining reaction was seen in 71% of the insufficient results (20 of 28). The majority of the laboratories was able to demonstrate PMS2 in cells with high-level antigen expression as proliferating germinal centre B-cells and basal epithelial cells of the appendix, whereas demonstration of PMS2 in cells with low-level antigen expression as resting mantle zone B-cells, smooth muscle and stromal cells could only be obtained by an optimally calibrated protocol (see Figs. 1-7). In this context, it has to be emphasized that identification of loss of PMS2 in tumours is characterized by a negative staining reaction of the neoplastic cells. Consequently, it is of decisive importance that the normal cells among neoplastic cells show a distinct positive nuclear staining reaction, serving as internal positive control. In the remaining 29% of the insufficient results, too weak specific staining reaction, poor signal to noise ratio or an excessive background staining (compromising interpretation) were seen.

28% (70 of 246) of the laboratories used Abs as Conc format within laboratory developed (LD) assays for PMS2. Optimal staining results could be obtained with the mAb clones A16-4, MRQ-28 and the rmAb clones EP51 and EPR3947 (see Table 1). Irrespective of the clone applied, careful calibration of the titre and efficient HIER at high pH were the main protocol prerequisites for optimal results. Both 2- and 3-step polymer/multimer based detection systems could be used to provide an optimal result, but the wast majority of laboratories (87%) used 3-step polymer/multimer based detection systems. The rmAb clone EP51 was the most widely used Ab for demonstration of PMS2 and provided a high proportion of sufficient staining results. Optimal results could be obtained on all four main IHC systems from Dako, Leica and Ventana (see Table 3), but especially high proportions of optimal staining results were seen on the Dako Omnis and Leica Bond platforms.

72% (176 of 246) of the laboratories used Abs in RTU formats. This was a minor increase compared to the previous PMS2 assessment in 2014, where 63% of the laboratories used the RTU format. The most widely used RTU systems for PMS2 were the rmAb clone EPR3947 based **760-4531** from Ventana/Roche, intended for use on the Ventana BenchMark System, and the rmAb clone EP51 based **IR087** from Dako/Agilent, intended for use on the Dako Autostainer System. In concordance with previous assessments, the proportion of sufficient staining results was high for both the 760-4531 and IR087 system. 88% (73 of 83) and 98% (40 of 41), respectivly, acheived sufficient staining results. 96% (80 of 83) of the laboratories using the rmAb clone EPR3947 based 760-4531 RTU system modified protocol

settings. Typically, laboratories used the 3-step multimer system OptiView (with or without amplification) instead of the recommended 2-step multimer system UltraView. Consequencely, the proportion of sufficient staining results increased from 67% to 89%. With the IR087 RTU system, the proportion of sufficient staining results was 100% (20 of 20) using the recommended protocol settings. This only dropped marginally to 95% (20 of 21) when modified protocol settings were used. The IR087 RTU system is developed and intended for use on the Dako Autostainer System, but 17 laboratories used the IR087 RTU within a laboratory developed (LD) assay for PMS2 on the Dako Omnis platform. All 17 laboratories made the same important modification to the recommeded "Autostainer-protocol", changing the 2-step EnVision Flex polymer detection system to the more sensitive 3-step EnVision Flex+ polymer detection system. Consequently, the proportion of sufficient staining results for this LD assay reached 100% with 76% (13 of 17) being optimal. These observations underline – once again – that RTU Abs used on other systems than the intended must be considered as a LD assay, thus requiring a thorough technical calibration and diagnostic validation before use in daily diagnostic practice.
The recently introduced mAb clone A16-4 based RTU system **790-5094** (Ventana/Roche), intended for use on the Ventana BenchMark System, also provided a pass rate of 100% (4 of 4).

This was the second assessment of PMS2 in NordiQC (see Table 2). The number of participants increased from 131 laboratories in 2014 to 246 laboratories in the current assessment. Despite this 88% increase in participating laboratories, the high pass rate from 2014 was not only maintained but slightly improved to 89% compared to 82% in run 41 in 2014. These data reflect the almost exclusive use of the highly recommendable rmAb clones EP51 and EPR3947 and the mAb clone A16-4.

Controls
Tonsil was found to be a recommendable positive tissue control for PMS2. 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 PMS2 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 stromal cells serving as internal positive tissue control.

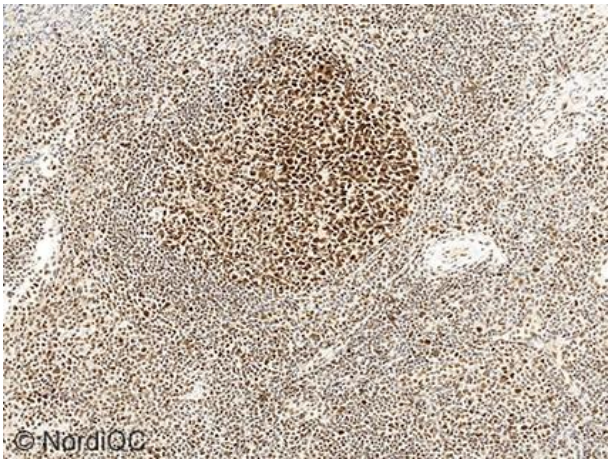


Fig. 1a
Optimal PMS2 staining reaction of the tonsil using the rmAb clone EP51 based IR087 RTU system for the Dako Autostainer platform following the recommended protocol settings – including 20 min. HIER in an alkaline buffer at 97°C. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Also compare with Figs. 2a - 4a, same protocol.

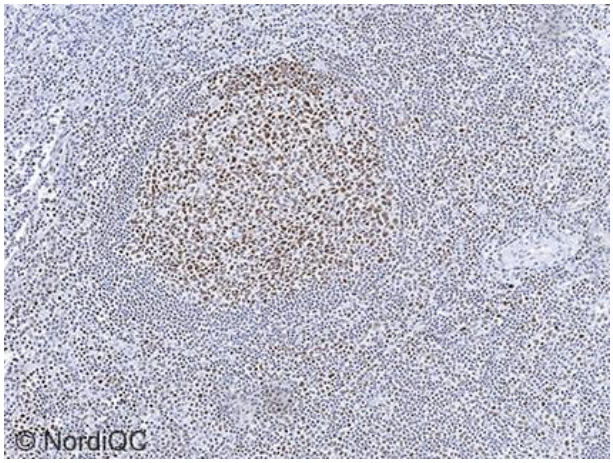


Fig. 1b
Insufficient PMS2 staining reaction of the tonsil using the rmAb clone EP51 based IR087 RTU system for the Dako Autostainer platform with a protocol providing too low analytic sensitivity - same field as in Fig. 1a. Only the germinal centre B-cells are distinctively demonstrated, while mantle zone B-cells expressing low levels of PMS2 virtually are unstained. This protocol was based on the recommended protocol settings but the HIER settings were modified. HIER for 1 min. in an alkaline buffer at 120°C was used. Too short HIER time in combination with excessive counter stain resulted in insufficient staining result. Also compare with Figs. 2b – 4b, same protocol.

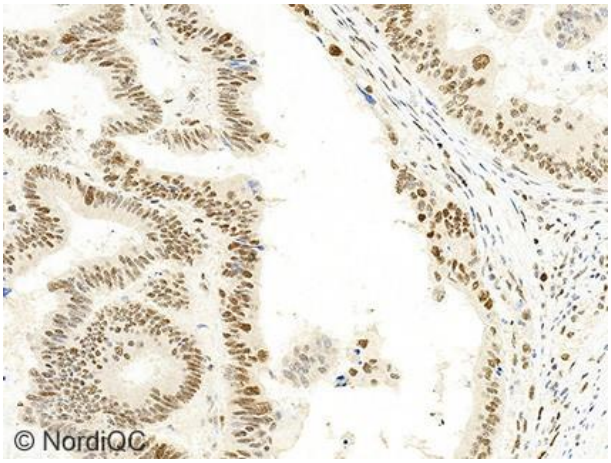


Fig. 2a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 3, with normal PMS2 expression using same protocol as in Fig. 1a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. A high signal-to-noise ratio is obtained with only faint cytoplasmic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

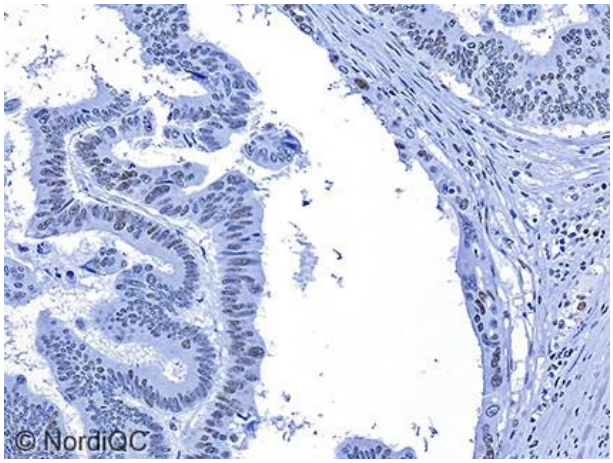


Fig. 2b
Insufficient PMS2 staining reaction for of the colon adenocarcinoma, tissue core no. 3, using same protocol as in Fig. 1b – same field as in Fig. 2a. The proportion and intensity of cells demonstrated are reduced compared to the result expected and shown in Fig. 2a. Also note that stromal cells virtually are negative. Too low analytical sensitivity in combination with excessive counterstain makes reliable interpretation difficult. Also compare with Figs. 3b – 4b, same protocol.

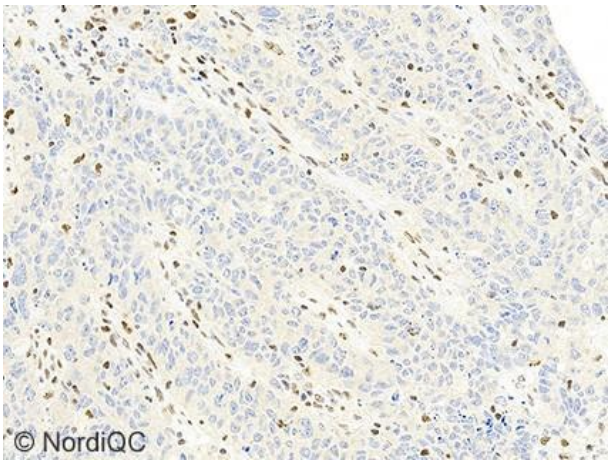


Fig. 3a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 5, with loss of PMS2 expression using same protocol as in Figs. 1a – 2a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

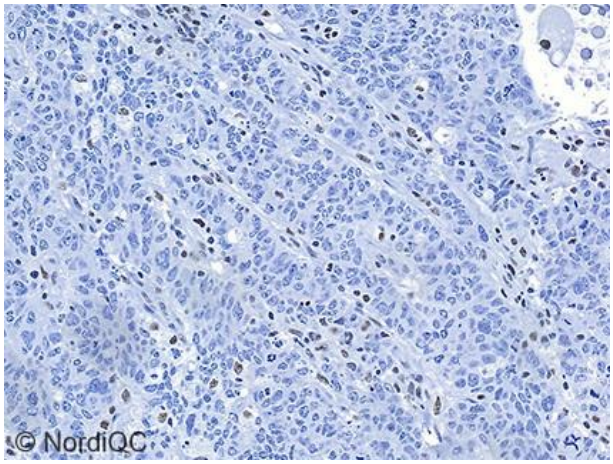


Fig. 3b
Insufficient PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 5, with loss of PMS2 expression using same protocol as in Figs. 1b – 2b – same field as in Fig. 3a. No nuclear staining reaction in the neoplastic cells is seen, but too low analytical sensitivity in combination with excessive counterstain makes reliable interpretation difficult.

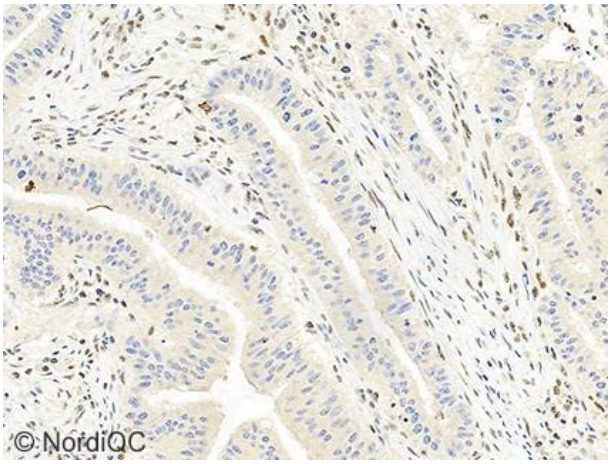


Fig. 4a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of PMS2 expression using same protocol as in Figs. 1a – 3a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. A faint cytoplasmic reaction is accepted.

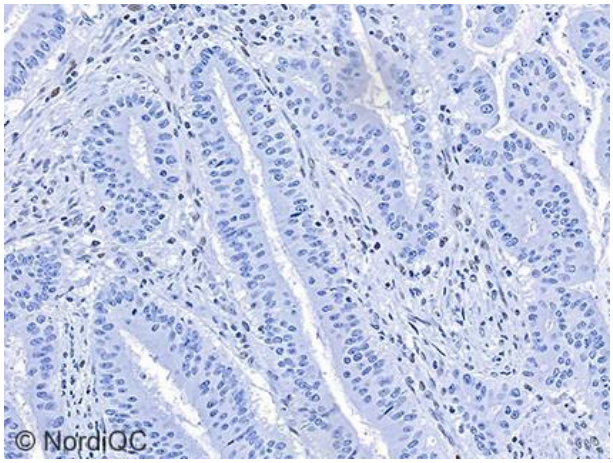


Fig. 4b
Insufficient PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of PMS2 expression using the same protocol as in Figs. 1b – 3b. Excessive counterstain in combination with too low analytical sensitivity makes reliable interpretation difficult. Only faint nuclear staining is seen in a few stromal cells serving as internal positive tissue control. Compare with Fig. 4a – same field.

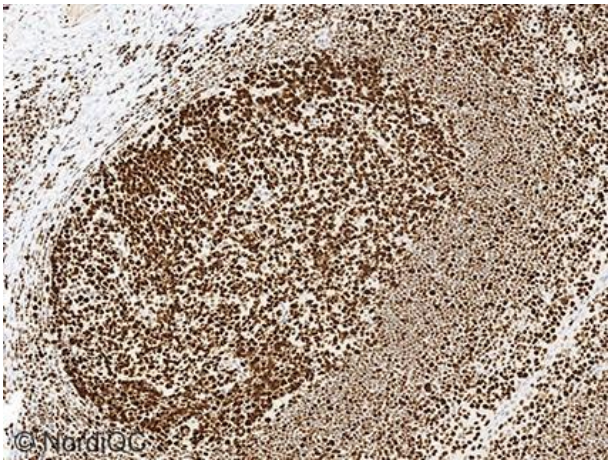


Fig. 5a
Optimal PMS2 staining reaction of the tonsil using the rmAb clone EPR3947 based 760-4531 RTU system for the Ventana BenchMark platform following laboratory modified protocol settings. HIER in CC1 for 64 min. and 32 min. incubation in the RTU Ab, followed by OptiView detection with an 8 min. amplification step. 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. 6a and 7a, same protocol.

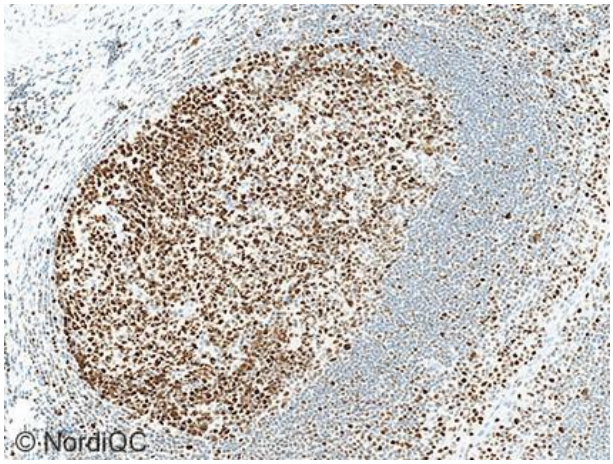


Fig. 5b
Insufficient PMS2 staining reaction of the tonsil using the rmAb clone EPR3947 based 760-4531 RTU system for the Ventana BenchMark platform following laboratory modified protocol settings simillar to Fig. 5a, but with a 50% reduction in HIER time, incubation of the primary Ab and amplification step. These modifications had a significant negative impact on the analytical sensitivity of the protocol. Only germinal centre B-cells are distinctively demonstrated, while mantle zone B-cells expressing low levels of PMS2 are virtually unstained. Also compare with Fig. 6b, same protocol.

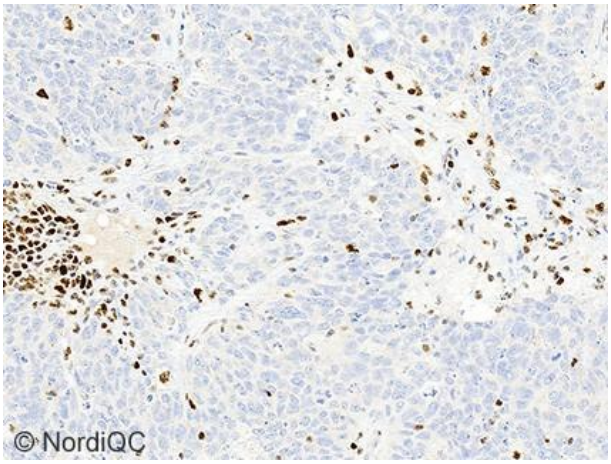


Fig. 6a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 5, with loss of PMS2 expression using same protocol as in Fig. 5a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

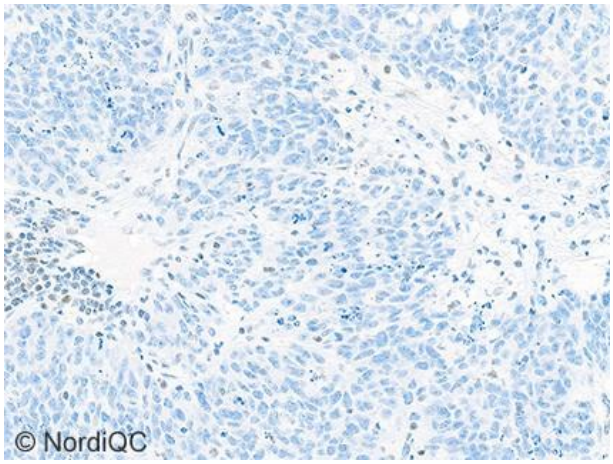


Fig. 6b
Insufficient PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 5, with loss of PMS2 expression using same protocol as in Fig. 5b – same field as in Fig. 6a. No nuclear staining reaction in the neoplastic cells is seen, but too low analytical sensitivity makes reliable interpretation difficult as only faint nuclear staining is seen in a few stromal cells serving as internal positive tissue control.

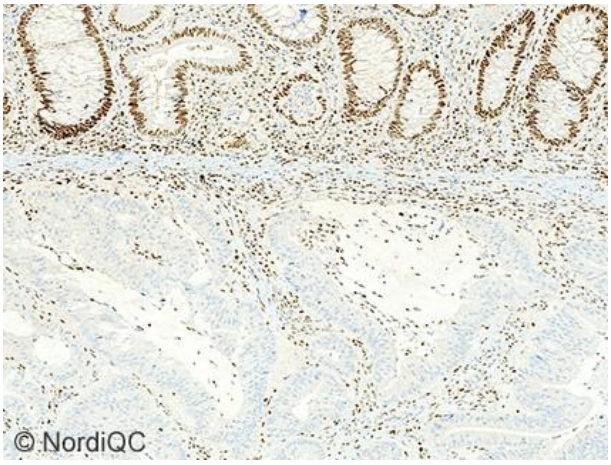


Fig. 7a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of PMS2 expression using same protocol as in Figs. 5a – 6a. The neoplastic cells are negative, while normal epithelial cells and stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

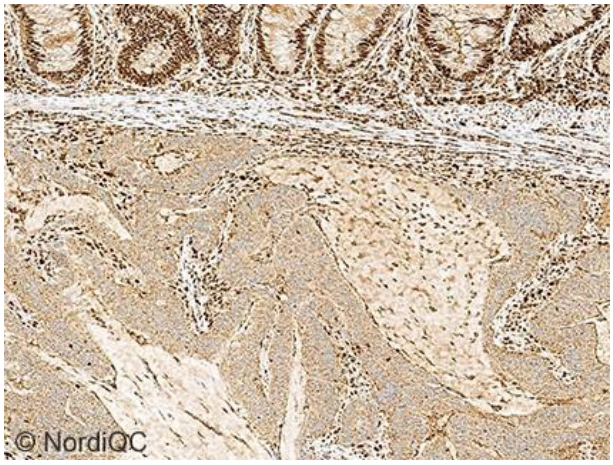


Fig. 7b
Insufficient PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of PMS2 expression using similar protocol setting as in Figs. 5a – 7a, except for a prolonged incubation time in primary Ab (120 min. compared to 32 min.). This results in strong cytoplasmic background staining in all cells, making interpretation very difficult – same field as in Fig. 7a.

ON/LE/MV/RR 12.06.18