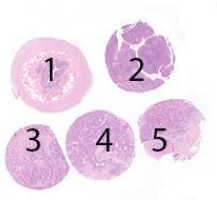
Assessment Run 62 2021

Mismatch repair protein PMS2 (PMS2)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for PMS2 status in colon adenocarcinomas. Loss of PMS2 function due to gene mutation or epigenetic changes is characterized by absence of nuclear expression in neoplastic cells, whereas intact nuclear PMS2 expression indicates normal PMS2 function and no gene mutations.

Material
The slide to be stained for PMS2 comprised:



1. Appendix, 2. Tonsil, 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 and stromal cells in the colon adenocarcinoma no. 3
- No nuclear staining reaction of the neoplastic cells in the colon adenocarcinomas no. 4 and 5, but an at least 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

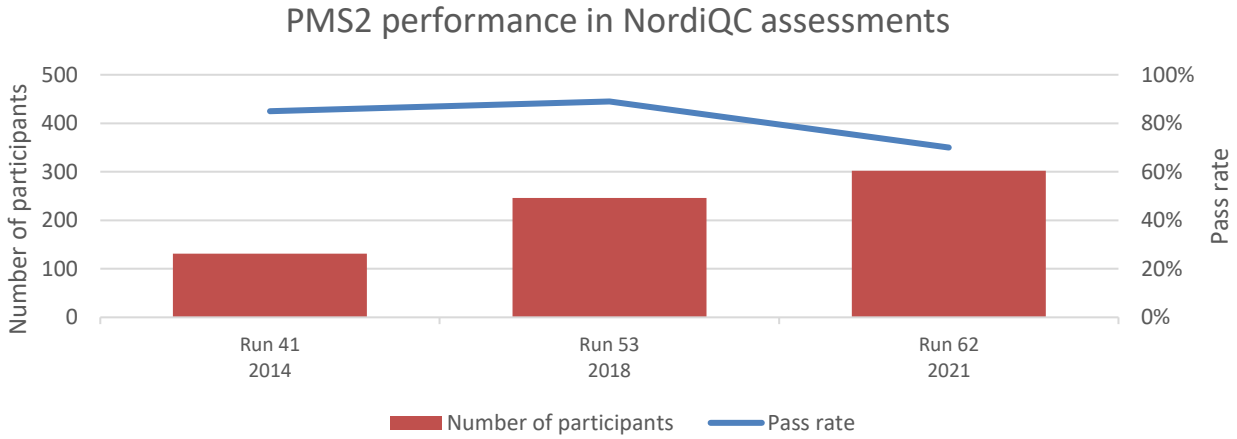
Number of laboratories registered for PMS2, run 62	322
Number of laboratories returning slides	302 (94%)

Results
302 laboratories participated in this assessment. 210 (70%) of these achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 3). All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

The most frequent causes of insufficient staining were:
- Less successful performance of the Ventana/Roche RTU format based on mAb clone A16-4
- Use of detection systems with too low sensitivity

Performance history
This was the third NordiQC assessment of PMS2. A decrease in the pass rate was seen compared to the previous runs.

Graph 1. **Proportion of sufficient results for PMS2 in the three NordiQC runs performed**



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.** Irrespective of the clone applied, efficient HIER (Heat Induced Epitope Retrieval) in an alkaline buffer, use of a sensitive polymer/multimer based 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/Agilent), Autostainer (Dako/Agilent), Bond (Leica Biosystems) and BenchMark (Ventana/Roche). Corresponding Ready-To-Use (RTU) systems based on rmAb clone EP51 from e.g. Dako/Agilent, Leica Biosystems and Sakura Finetek also provided a high pass rate and proportion of optimal results. Contrary to these, the Ventana/Roche RTU system based on mAb clone A16-4 showed an inferior performance.

Tonsil is recommendable as positive tissue control for PMS2. Virtually all 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 62**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff[1]	OR[2]
mAb clone **A16-4**	8 2 2	BD Biosciences Zytomed Bicare	6	5	0	1	92%	50%
mAb clone **M0R4G**	1	Leica Biosystems	0	1	0	0	-	-
mAb clone **MRQ-28**	1	Cell Marque	1	0	0	0	-	-
rmAb clone **EP51**	23 6 2 6 1 1 1	Dako/Agilent Epitomics Nordic Biosite Cell Marque Abcam Master Diagnostica Zeta Corporation	30	7	3	0	93%	75%
rmAb clone **EPR3947**	3	Abcam	2	1	0	0	-	-
Ab **QR009**	2	Quartett	0	0	2	0	-	-
Ready-To-Use antibodies								
mAb clone **A16-4 PM344AA**	4	BioCare	3	1	0	0	**-**	**-**
mAb clone **A16-4 PDM171**	2	Diagnostic Biosystems	0	1	1	0	-	-
mAb clone **A16-4 790-5094 (VRPS)[3]**	33	Ventana/Roche	5	11	17	0	49%	15%
mAb clone **A16-4 790-5094 (LMPS)[4]**	86	Ventana/Roche	11	22	42	11	38%	13%
mAb clone **MRQ-28 288M-18**	2	Cell Marque	1	0	1	0	**-**	**-**
rmAb clone **EP51 IR087 (VRPS)[3]**	3	Dako/Agilent	2	0	0	1	-	-
rmAb clone **EP51 IR087 (LMPS)[4]**	51	Dako/Agilent	26	17	7	1	84%	51%
rmAb clone **EP51 GA087 (VRPS)[3]**	22	Dako/Agilent	18	4	0	0	100%	82%
rmAb clone **EP51 GA087 (LMPS)[4]**	12	Dako/Agilent	9	1	2	0	83%	75%
rmAb clone **EP51 PA0991 (VRPS)[3]**	3	Leica Biosystems	3	0	0	0	-	-
rmAb clone **EP51 PA0991 (LMPS)[4]**	4	Leica Biosystems	2	1	1	0	-	-
rmAb clone **EP51 MAD-000681QD**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **EP51 8328-C010**	4	Sakura Finetek	4	0	0	0	-	-
rmAb clone **EP51 RMA-0775**	2	Fuzhou Maixin	2	0	0	0	-	-
rmAb clone **EP51 CPR-0321**	1	Celnovte	1	0	0	0	-	-
rmAb clone **EPR3947 288R-10/17/18**	8	Cell Marque	3	4	1	0	88%	38%
rmAb clone **EPR3947 760-4531**	2	Ventana/Roche	0	2	0	0	-	-
rmAb clone **EPR3947 Ab214442**	1	Abcam	0	1	0	0	-	-
rmAb clone **IHC422 IHC422**	1	GenomeMe	0	1	0	0	-	-
Total	302		129	81	78	14		
Proportion			43%	27%	26%	4%	70%	

1) Proportion of sufficient stains (optimal or good) (≥5 asessed protocols).
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

Detailed analysis of PMS2, Run 62
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 Biosystems) (6/9)* as retrieval buffer. The mAb was diluted in the range of 1:30-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 9 of 9 (100%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP51**: Protocols with optimal results were all based on HIER using BERS2 (Leica Biosystems) (9/10), Cell Conditioning Solution 1 (CC1, Ventana/Roche) (13/16), Target Retrieval Solution (TRS) High pH (Dako/Agilent) (6/11) or Tris-EDTA/EGTA pH 9 (2/2) as retrieval buffer. The rmAb was typically diluted in the range of 1:20-1:400 depending on the total sensitivity of the protocol employed. Using these protocol settings, 37 of 39 (95%) laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for PMS2 for the most commonly used antibodies as concentrates on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer Link / Classic**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark GX / XT / Ultra**		**Leica Biosystems 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	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **A16-4**	-	-	-	-	-	-	6/9** (67%)	-
rmAb clone **EP51**	2/4 (50%)	-	4/7 (57%)	-	13/16 (81%)	-	9/10 (90%)	-

* 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. **790-5094**, Ventana/Roche, Ventana Benchmark GX/XT/Ultra:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time for 72-96 min. at 100°C), 12-60 min. incubation at 36°C of the primary Ab and OptiView (760-700) with tyramide signal amplification (760-099 / 860-099) as detection system. Using these protocol settings, 38 of 85 (45%) laboratories produced a sufficient staining result (optimal or good).

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 High pH (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+ (K8000/K8002) as detection system. Using these protocol settings, 22 of 26 (85%) laboratories produced a sufficient staining result (optimal or good).
26 laboratories used product no. IR087 on other platforms. These were not included in the description above.

rmAb clone **EP51**, product no. **GA087**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 30 min.), 20 min. incubation of the primary Ab and EnVision FLEX+/FLEX++ (GV800/GV823/GV809/GV821) as detection system. Using these protocol settings, 28 of 28 (100%) laboratories produced a sufficient staining result (optimal or good).

rmAb clone **EP51**, product no. **PA0991**, Leica Biosystems, Bond III/Max:
Protocols with optimal results were based on HIER using BERS2 for 20-30 min., 15-30 min. incubation of the primary Ab and Bond Refine (DS9800) as detection system. Using these protocol settings, 5 of 6 (83%) laboratories produced a sufficient staining result (optimal or good).

Table 3 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 3. **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**	16/33 (48%)	5/33 (15%)	33/86 (38%)	11/86 (13%)
Dako AS rmAb clone **EP51, IR087**	2/3	2/3	21/25 (84%)	17/25 (68%)
Dako Omnis rmAb clone **EP51, GA087**	22/22 (100%)	18/22 (81%)	6/8 (75%)	6/8 (75%)
Leica Bond rmAb clone **EP51, PA0991**	3/3	3/3	2/3	2/3

* Protocol settings recommended by vendor – retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Comments

In this assessment the prevalent feature of an insufficient staining reaction was a poor signal-to-noise ratio and/or an excessive background staining complicating the interpretation. This pattern was seen in 71% (65 of 92) of the insufficient results. In 27% (25 of 92) of the insufficient results, too weak or completely false negative staining reactions were seen. In the remaining 2% a false positive nuclear staining reaction in tumors with loss of PMS2 expression (tissue cores no. 4-5) was observed.

20% (59 of 302) 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 (86%) used 3-step polymer/multimer based detection systems obtaining a pass rate of 94% compared to laboratories using 2-step polymer/multimer based detection system with a pass rate of 67%. The rmAb clone EP51 was the most widely used Ab for demonstration of PMS2 and provided a high proportion of sufficient and optimal staining results. Optimal results could be obtained on all four main IHC systems from Dako/Agilent, Leica Biosystems and Ventana/Roche (see Table 2), but especially high proportions of optimal staining results were seen on the BenchMark and Bond platforms.

80% (243 of 302) of the laboratories used Abs in RTU formats. The use of RTUs for PMS2 has increased from 63% in 2014 and 72% in 2018.

The most widely used RTU system for PMS2, Ventana/Roche **790-5094** based on mAb clone A16-4, being used by 38% of all participants with intended use on the BenchMark systems, provided a pass rate of 49% if using the vendor recommended protocol settings and 38% if modifying the protocol (see Table 3). The majority of insufficient results were caused by a poor signal-to-noise ratio and/or excessive cytoplasmic staining reaction complicating the interpretation. Extensive data analysis to identify the root cause(s) for the aberrant results did not provide any specific explanations for the different staining results and lack of reproducibility using similar protocol settings and same primary Ab lots by a great number of laboratories. As no plausible explanations correlated to the protocols applied could explain the aberrant results, and thus it is most likely caused by "technical system issues" related to e.g. the instruments, reagents incl. the amplification kit or other general components of the IHC test. Participants obtaining an insufficient result were recommended to "Repeat the protocol and verify protocol settings".

The newly introduced RTU system **GA087** from Dako/Agilent, based on rmAb clone EP51 with intended use on the Dako Omnis system, gave a pass rate at 100% when following the vendor recommended protocol settings and 83% when modifying the protocol (see Table 3). The recommended protocol use dual linker in the detection system. Two laboratories used the GA087 with Envision Flex detection system without linkers, giving false negative staining results. If applying at least one linker, all participants obtained sufficient staining results.

The **IR087** RTU system from Dako/Agilent based on rmAb EP51, intended use on the Dako Autostainer systems, gave an overall pass rate at 83%. Only three laboratories followed the vendor recommended protocol settings, whereas the protocols most frequently were adjusted by prolonging incubation time of primary Ab and/or using EnVision Flex+ as detection system.

The RTU system **PA0991** from Leica Biosystems based on rmAb clone EP51, intended use on the Bond systems, achieved an overall pass rate at 86%. If following the recommended protocol settings, three of three obtained optimal score, and if modifying the protocol settings (prolonging efficient HIER time) two of three passed with an optimal score.

This was the third assessment of PMS2 in NordiQC (see Graph 1). The number of participants increased from 131 laboratories in 2014 to 246 in 2018 to 302 in the current assessment. The decrease in the pass rate was most likely caused by a less succesfull performance of the Venana/Roche RTU system based on the mAb clone A16-4 (790-5094). In this context it was observed that, the Ventana RTU system based on rmAb clone EPR3947 (760-4531) in the latest PMS2 assessment gave a pass rate of 88%.

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.

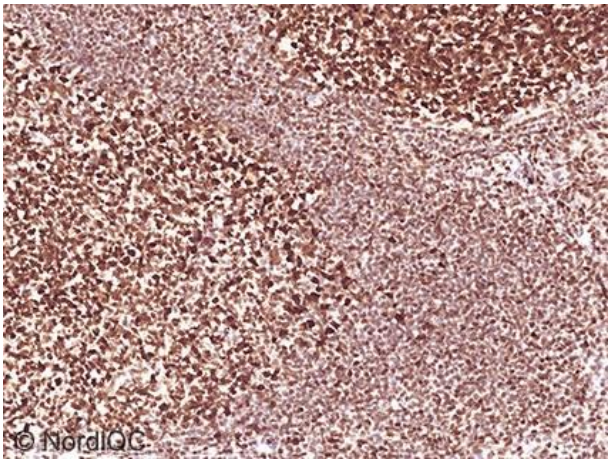


Fig. 1a
Optimal PMS2 staining reaction of the tonsil using the GA087 RTU system for the Dako Omnis platform based on rmAb clone EP51, following the recommended protocol settings using dual linker. Virtually all mantle zone B-cells show a moderate and distinct nuclear staining reaction, while the germinal centre B-cells show a strong nuclear staining reaction. Also compare with Figs. 2a - 5a, same protocol.

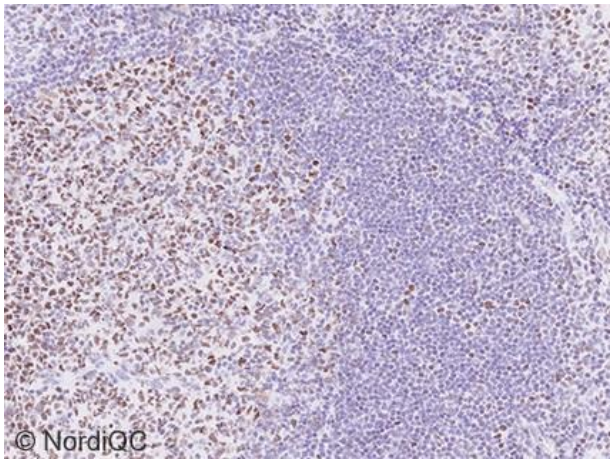


Fig. 1b
Insufficient PMS2 staining reaction of the tonsil using the rmAb clone EP51 on the Dako Omnis platform with a protocol providing too low analytic sensitivity - same field as in Fig. 1a. Only the germinal centre B-cells are distinctively demonstrated, while mantle zone B-cells expressing low levels of PMS2 are virtually unstained. This protocol was based on EnVision Flex without linker(s) resulteing in an insufficient staining result. Also compare with Figs. 2b – 5b, same protocol.

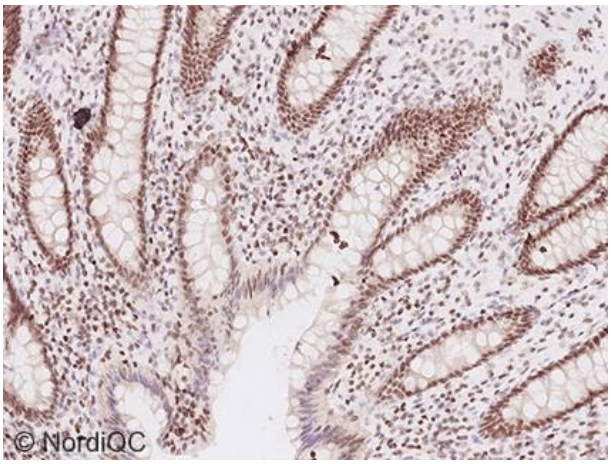


Fig. 2a
Optimal PMS2 staining reaction of the appendix using same protocol as in Fig. 1a. Virtually all cells show an at least weak nuclear staining reaction. Also compare with Figs. 3a - 5a, same protocol.

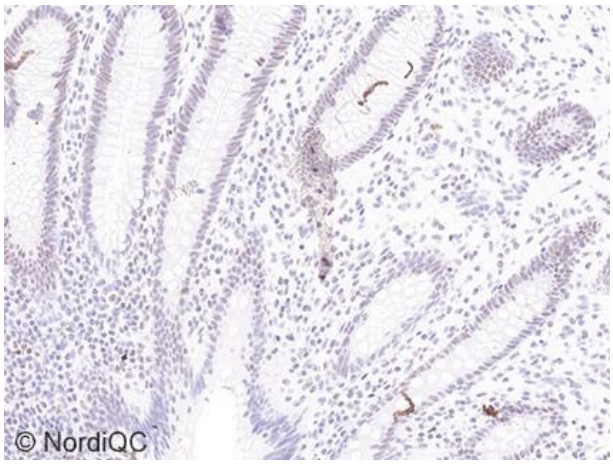


Fig. 2b
Insufficient PMS2 staining reaction of the appendix using same protocol as in Fig. 1b – same field as in Fig. 2a. Only a few cells show a weak nuclear staining reaction. Also compare with Figs. 3b – 5b, same protocol.

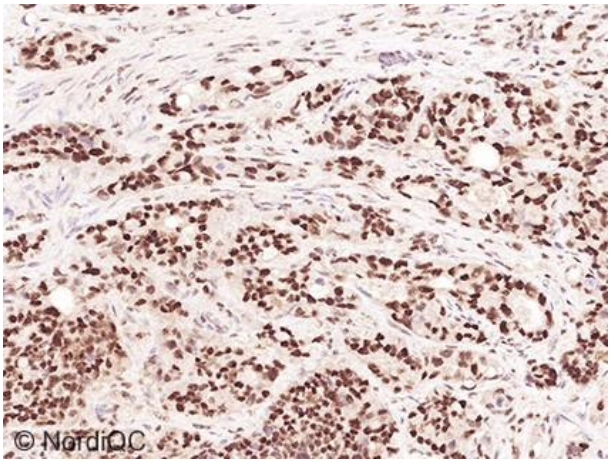


Fig. 3a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 3, with normal PMS2 expression using same protocol as in Figs. 1a – 2a. Virtually all neoplastic cells show a moderate to strong nuclear staining reaction with only faint cytoplasmic staining.

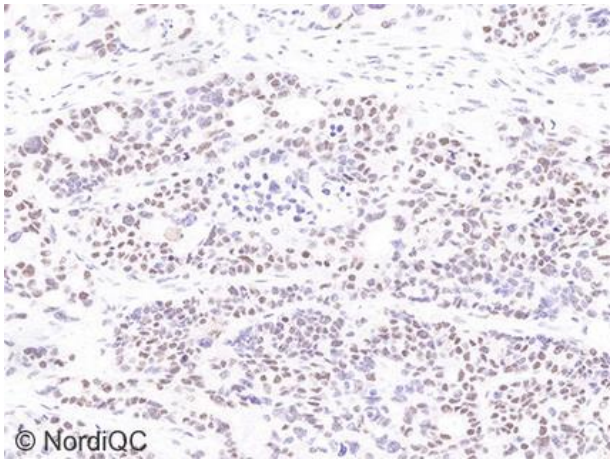


Fig. 3b
Insufficient PMS2 staining reaction for of the colon adenocarcinoma, tissue core no. 3, using same protocol as in Figs. 1b – 2b, same field as in Fig. 3a.
The proportion and intensity of cells demonstrated are reduced compared to the result expected and shown in Fig. 3a. Also note that stromal cells virtually are negative. Also compare with Figs. 4b – 5b, same protocol.

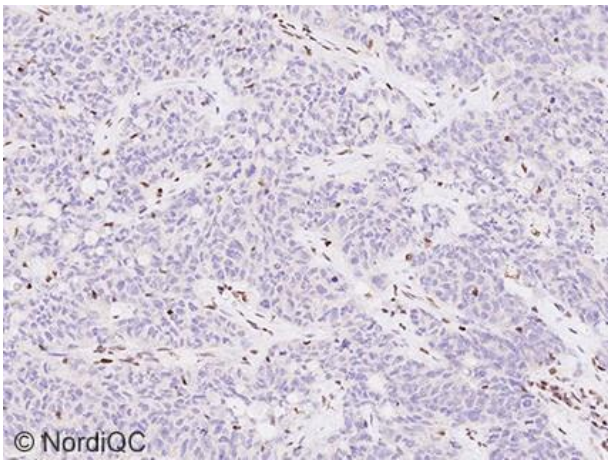


Fig. 4a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of PMS2 expression using same protocol as in Figs. 1a – 3a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control.

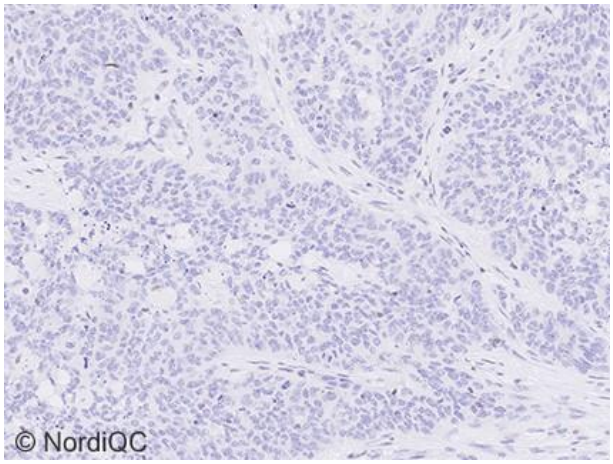


Fig. 4b
Insufficient PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 4, with loss of PMS2 expression using same protocol as in Figs. 1b – 3b, same field as in Fig. 4a. No nuclear staining reaction in the neoplastic cells is seen, but the too low analytical sensitivity and hereby almost negative result in stromal cells in combination with excessive counterstain compromises the interpretation.

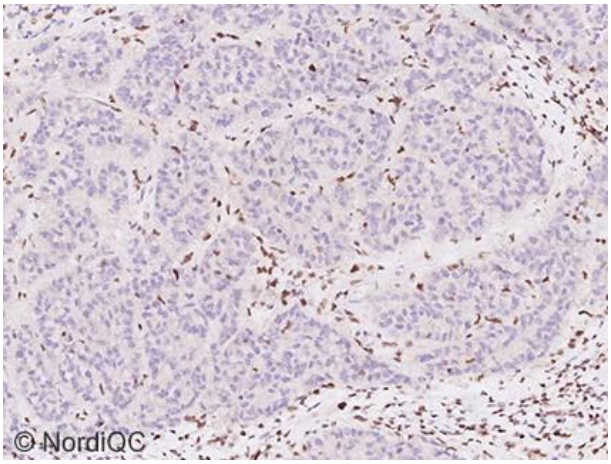


Fig. 5a
Optimal PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 5, with loss of PMS2 expression using same protocol as in Figs. 1a – 4a. The neoplastic cells are negative, while stromal cells show a distinct nuclear staining reaction serving as internal positive tissue control. A faint cytoplasmic reaction is accepted.

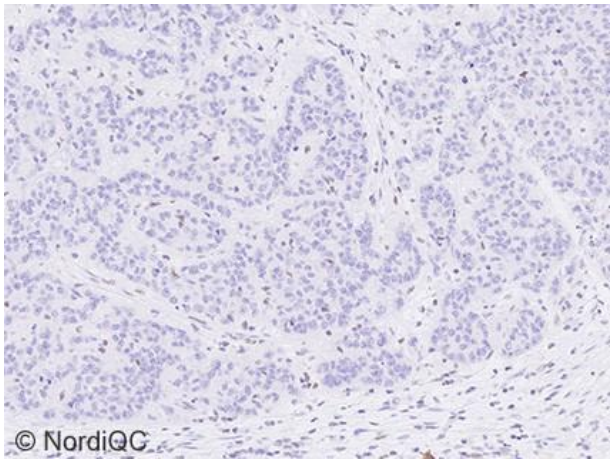


Fig. 5b
Insufficient PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 5, with loss of PMS2 expression using the same protocol as in Figs. 1b – 4b. Only faint nuclear staining is seen in a few stromal cells, serving as internal positive tissue control, compromising the interpretation. Compare with Fig. 5a – same field.

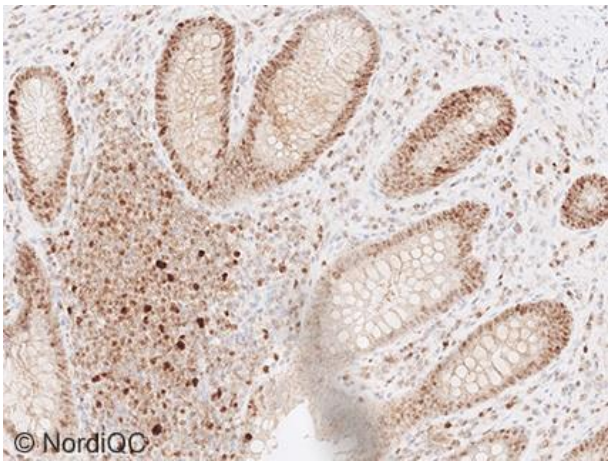


Fig. 6a
Insufficient PMS2 staining reaction of the appendix using the mAb clone A16-4, 790-5094 RTU system for the Ventana BenchMark platform following vendor recommended protocol settings. A poor signal-to-noise ratio was seen, giving a cytoplasmic staining reaction especially in the epithelial cells. Compare with Fig. 2a for optimal result – same area. Also compare with Fig. 6b same protocol.

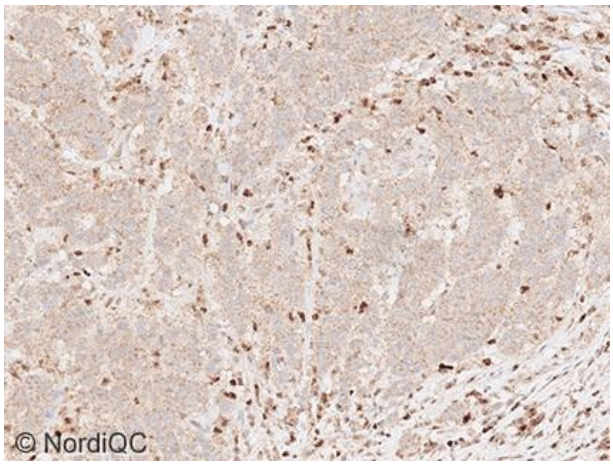


Fig. 6b
Insufficient PMS2 staining reaction of the colon adenocarcinoma, tissue core no. 5, with loss of PMS2 expression using the same protocol as in Fig. 6a. A poor signal-to-noise ratio giving a weak cytoplasmic and nuclear staining complicating the interpretation of the neoplastic cells expected to be negative. Compare with Fig. 5a for optimal result – same area.

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