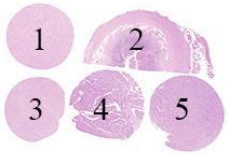
Assessment Run 62 2021
BRAF (BRAF V600E)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of BRAF IHC tests among NordiQC participants for the demonstration of corresponding BRAF V600E mutations in melanomas and colorectal adenocarcinomas.

Material
The slide to be stained for BRAF comprised:



1. Malignant melanoma with BRAF V600E mutation*, 2. Appendix, 3. Malignant melanoma without BRAF V600E mutation*, 4. Colon adenocarcinoma without BRAF V600E mutation*, 5. Colon adenocarcinoma with BRAF V600E mutation*.

*BRAF V600E mutation status confirmed by Next Generation Sequencing (NGS).

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a BRAF staining as optimal included:

- An at least weak to moderate distinct cytoplasmic staining reaction in virtually all neoplastic cells in the malignant melanoma tissue core no. 1 and colon adenocarcinoma tissue core no. 5.
- No staining reaction in neoplastic cells in the malignant melanoma tissue core no. 3 and colon adenocarcinoma tissue core no. 4.
- No cytoplasmic staining reaction in epithelial cells in appendix. A weak staining reaction in the smooth muscle layer and nuclear staining reaction in epithelial cells was accepted.

Participation

Number of laboratories registered for BRAF, run 62	147
Number of laboratories returning slides	135 (92%)

All slides returned after the assessment will be assessed and receive advice if the result is insufficient but will not be included in this report.

Results
135 laboratories participated in this assessment. 97 (72%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks given (see page 2).

The most frequent causes of insufficient staining reactions were:
- Less successful primary antibodies
- Less successful performance of the mAb clone VE1 on other platforms than Ventana/Roche BenchMark platforms.

Performance history
This was the first NordiQC assessment of BRAF and the overall pass rate was 72% (see Table 2).

Table 2. **Proportion of sufficient results for BRAF in the first NordiQC run performed**

	Run 62 2021
Participants, n=	135
Sufficient results	72%

Conclusion
In this first NordiQC assessment of BRAF, optimal staining results could only be obtained with the mouse monoclonal Ab (mAb) clone **VE1** both applied as concentrated format and RTU system on the Ventana/Roche BenchMark platforms. The Ventana/Roche RTU system provided a higher pass rate compared to laboratory developed assays based on the corresponding concentrate of clone VE1.
Optimal results were mainly obtained by using efficient HIER in an alkaline buffer and use of a highly sensitive multimer based detection system with tyramide amplification.
No optimal results were obtained on Omnis (Dako/Agilent) and Bond (Leica Biosystems) platforms.

Tumors confirmed with and without BRAF V600E mutation are recommended as positive and negative tissue controls for BRAF. Appendix can also serve as a negative tissue control, where no staining reaction should be seen in the epithelial cells. At present, no data is available on consistent low-level expressing normal tissues/cells, and thus it is important to secure a distinct and an "as strong as possible reaction" for BRAF in mutated tumors and still no reaction in negative tissue controls.

Table 1. **Antibodies and assessment marks for BRAF, run 62**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb **1H12***	1	Invitrogen	-	-	-	1	-	-
rmAb **EP152Y***	1	Abcam	-	-	-	1	-	-
rmAb **RM8**	2	RevMAb Biosciences	-	-	-	2	-	-
mAb **IHC600**	2	Dianova	-	-	1	1	-	-
mAb **VE1**	24 4	Abcam Spring Bioscience	9	5	12	2	50%	32%
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb **VE1** **760-5095 (VRPS)[3]**	23	Ventana/Roche	3	16	4	-	83%	13%
mAb **VE1** **760-5095 (LMPS)[4]**	72	Ventana/Roche	35	27	9	1	86%	49%
mAb **IHC600,** **IHC600**	2	GenomeMe	-	-	2	-	-	-
rmAb **RM8** **CBR-100**	1	Celnovte	-	-	-	1	-	-
rmAb **RM8** **RMA-0839**	2	Fuzhou Maixin	-	2	-	-	-	-
rmAb **V22-E** **DB 187-RTU**	1	Histo-line	-	-	-	1	-	-
Total	135		47	50	28	10		
Proportion			35%	37%	21%	7%	72%	

1) Proportion of sufficient stains (optimal or good). (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).
*) Not against the V600E mutation.

Detailed analysis of BRAF Run 62

The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies

mAb clone **VE1**: Protocols with optimal results were typically based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1, Ventana/Roche) (8/15)* or Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (1/3) as retrieval buffer. The mAb was typically diluted in the range of 1:50-2000 depending on the total sensitivity of the protocol employed. Using these protocol settings, 11/18 (61%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

Table 3. **Proportion of optimal results for BRAF for the most commonly used antibody as concentrate 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	ER2 pH 9.0	ER1 pH 6.0
mAb clone **VE1**	1/3**	-	0/7	-	8/15 (53%)	-	0/2	-

* 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 **VE1**, product no. **760-5095**, Ventana/Roche:
Protocols with optimal results were typically based on HIER using CC1 as retrieval buffer (efficient HIER time 48-64 min.), 16-32 min. Ab incubation and OptiView with or without OptiView Amplification as detection system. Using these protocol settings, 72/78 (92%) 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 BRAF for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS mAb clone **VE1, 760-5095**	19/23 (83%)	3/23 (13%)	60/67 (90%)	34/67 (51%)

* 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 first NordiQC assessment of BRAF, a pass rate of 72% was obtained.

The prevalent feature of an insufficient result of BRAF V600E mutation was characterized by too weak or completely false negative staining results, seen in 53% of the insufficient results (20 of 38). The majority of all laboratories were able to demonstrate BRAF V600E mutation in the malignant melanoma with verified BRAF V600E mutation, tissue core no. 1, but only antibodies and protocols with high level of analytical sensitivity managed to demonstrate BRAF V600E mutation in the colon adenocarcinoma, tissue core no. 5, with verified V600E mutation. 29% of the insufficient staining results were caused by a poor signal-to-noise ratio, especially seen in the neoplasias without BRAF V600E mutation, complicating the interpretation. A false positive staining reaction in one or both of the two neopasias without V600E mutation was seen in the remaining 18% of the insufficient staining results (7 of 38).

25% (34 of 135) of the laboratories used Abs as Conc. format within LD assay for BRAF mutation. Sufficient and optimal staining results were only obtained with the mAb clone VE1. The Ventana BenchMark platforms were both the most widely used and successful platforms. 88% of the optimal results (7 of 8) obtained on BenchMark were based on HIER in CC1, OptiView with OptiView amplification kit (tyramide based) as detection system, and an Ab titre in the range of 1:50-2.000. If same protocol settings were applied for the LD assay on BenchMark but omitting OptiView amplification, 13% (1 of 8) of the results were optimal.
Other Abs generated insufficient results typically being characterized by poor signal-to-noise ratio or false positive staining reaction in the non-BRAF mutated neoplasia's. The latter caused by use of Abs raised towards the full-length and native BRAF protein as rmAb clone EP152Y and not raised towards the V600E mutation related protein.

75% (101 of 135) of the laboratories used Abs in RTU formats.

The most widely used RTU system for BRAF, Ventana/Roche **760-5095** based on mAb clone VE1, being used by 70% of all participants with intended use on the BenchMark systems, provided a pass rate of 83% (13% optimal) if using the vendor recommended protocol settings and 90% (51% optimal) if modifying the protocol (see Table 4). The majority of insufficient staining results were caused by a too weak or completely false negative staining reaction in one or both of the neoplasias with BRAF V600E mutation, expected to display an at least weak cytoplasmic staining reaction. 58% of the laboratories modifying the protocol (39 of 67), added OptiView Amplification kit to the detection system of which 95% obtained an optimal score (37 of 39). In this first assessment of BRAF in NordiQC, the protocols based on OptiView Amplification kit facilitated the interpretation as the positive cytoplasmic staining reaction in the two neoplasias with BRAF V600E mutation was enhanced and still leaving the non-BRAF V600E mutated tumours negative. However it is well-known from previously NordiQC assessments, that assays based on OpiView Amplification kit (tyramide based) can be challenging to calibrate and frequently will provide a binary result as either negative or positive and not giving a "normal" dynamic range of antigen expression levels from low to high. The "lack" of dynamic range and the binary pattern can compromise the demonstration of low-level antigen expressing structures and at the same time also induce a risk of aberrant granular precipitation of the chromogen in structures expected to be negative. Nevertheless, the data observed in this assessment provided a higher proportion of optimal results for protocols based on OptiView with Amplification kit compared to standard OptiView detection system, whereas the overall pass rates were almost similar.
In this context it has to be emphasized that modifications of vendor clinically validated assays must be meticulously re-validated by the end-users on a large cohort of relevant tissue samples including attention to scoring guidelines for positive and negative read-out .

Controls
Tumors confirmed with and without BRAF V600E mutation are recommended as positive and negative tissue controls for BRAF. Appendix can also serve as a negative tissue control, where no staining reaction should be seen in the epithelial cells. At present, no data is available on consistent low-level expressing normal tissues/cells, and thus it is important to secure distinct and an "as strong as possible reaction" for BRAF in mutated tumors and still no reaction in negative tissue controls.

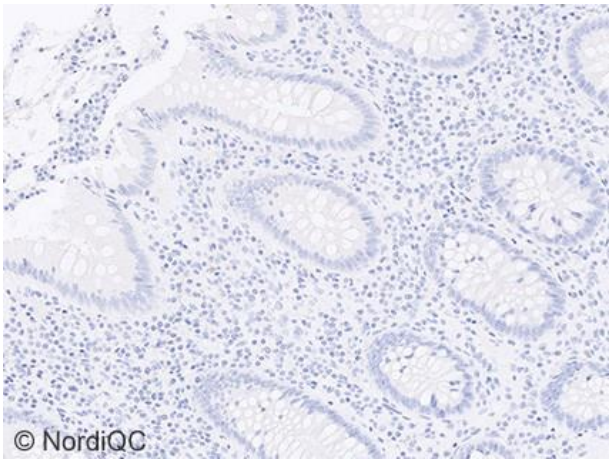


Fig. 1a
Optimal BRAF staining of the epithelial cells in appendix using the Ventana RTU 760-5095 based on mAb clone VE1, using the recommended protocol settings on Ventana BenchMark Ultra. No staining reaction is seen. Same protocol used in Figs. 2a-4a.

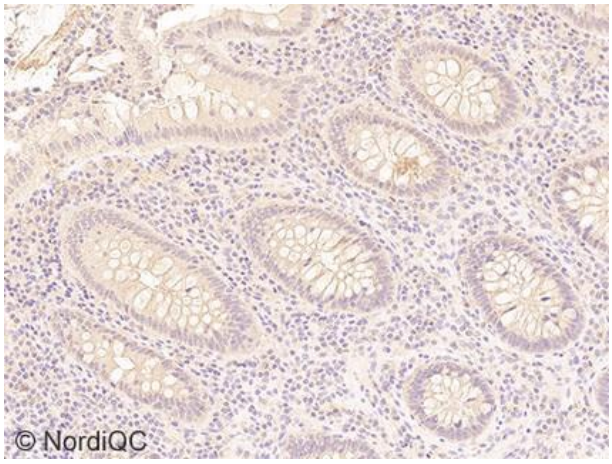


Fig. 1a
Insufficient BRAF staining of the epithelial cells in appendix using the mAb clone VE1, diluted, 1:100, epitope retrieval using HIER in an alkaline buffer (20 min.) and a 3-step multimer based detection system. An excessive background staining is seen. Compare with optimal results in Fig. 1a. Same protocol used in Figs. 2b-4b.

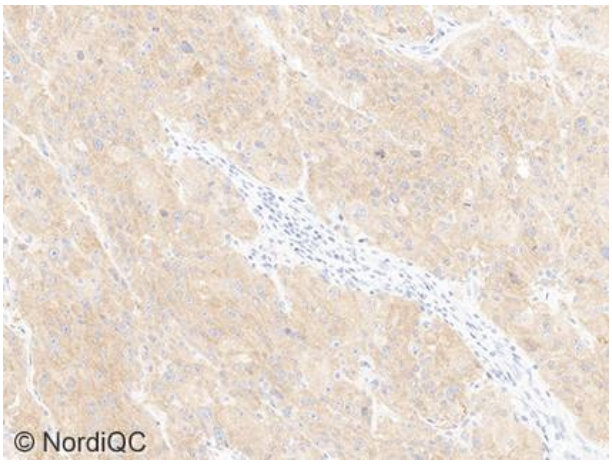


Fig. 2a
Optimal BRAF staining of the malignant melanoma with BRAF V600E mutation, tissue core no 1, using same protocol as in Fig. 1a. The neoplastic cells display a weak to moderate cytoplasmic staining reaction.

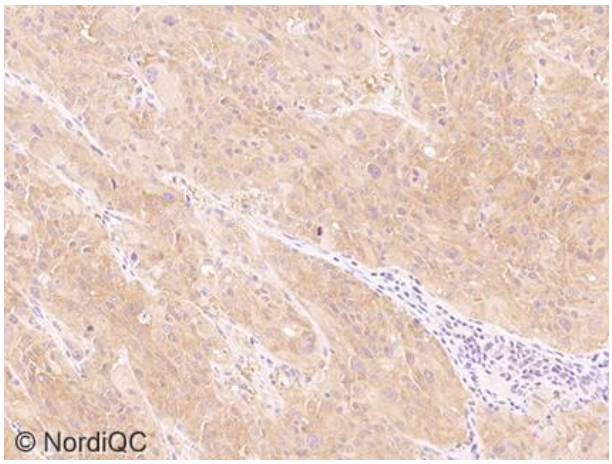


Fig. 2b
BRAF staining of the malignant melanoma with BRAF V600E mutation, tissue core no 1, using same insufficient protocol as Fig. 1b. The neoplastic cells display a moderate cytoplasmic staining reaction.

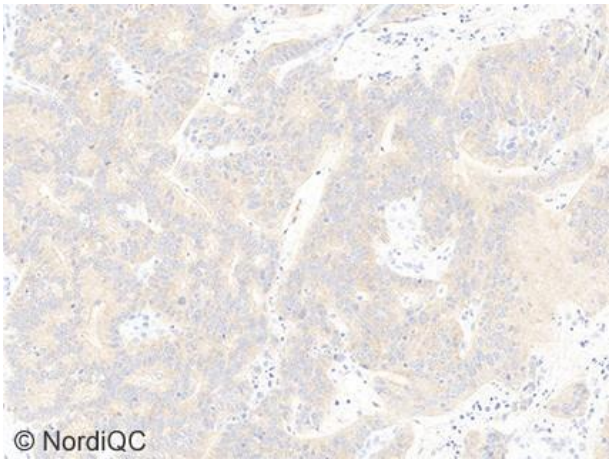


Fig. 3a
Optimal BRAF staining of the colon adenocarcinoma with BRAF V600E mutation, tissue core no. 5, using same protocol as in Figs. 1a – 2a. The neoplastic cells display a weak cytoplasmic staining reaction.

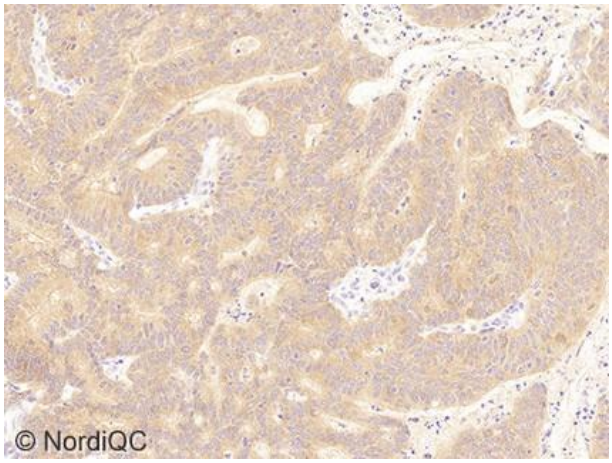


Fig. 3b
BRAF staining of the colon adenocarcinoma with BRAF V600E mutation, tissue core no. 5, using same insufficient protocol as in Figs. 1b – 2b. The neoplastic cells display a moderate staining reaction. A weak background staining is seen in the stromal compartment.

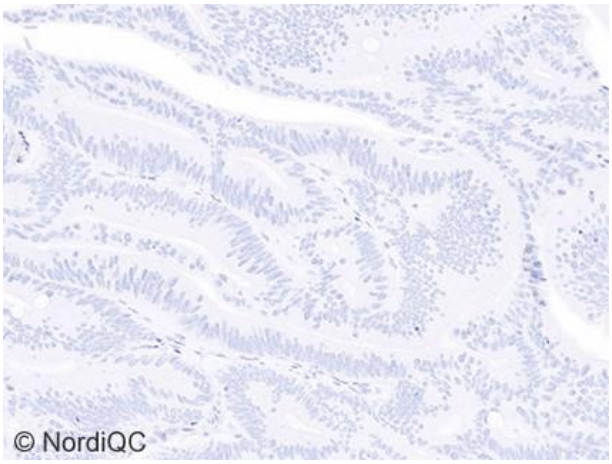


Fig. 4a
Optimal BRAF staining of the colon adenocarcinoma without BRAF V600E mutation, tissue core no. 4, using same protocol as in Figs. 1a – 3a. All cells are negative.

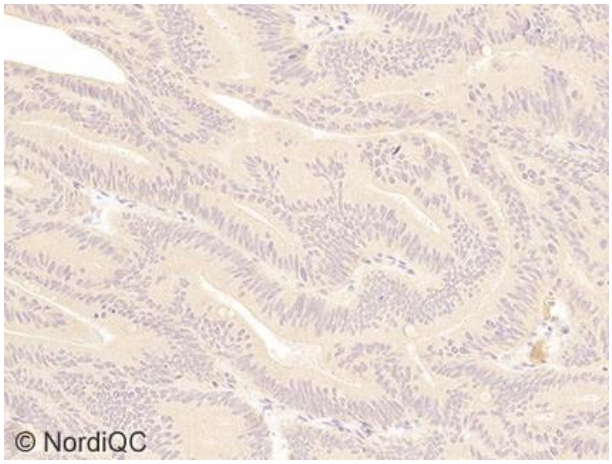


Fig. 4b
Insufficient BRAF staining of the colon adenocarcinoma without BRAF V600E mutation, tissue core no. 4, using same protocol as in Figs. 1b – 3b. The neoplastic cells show a false positive staining reaction. Compare with optimal result in Fig. 4a.

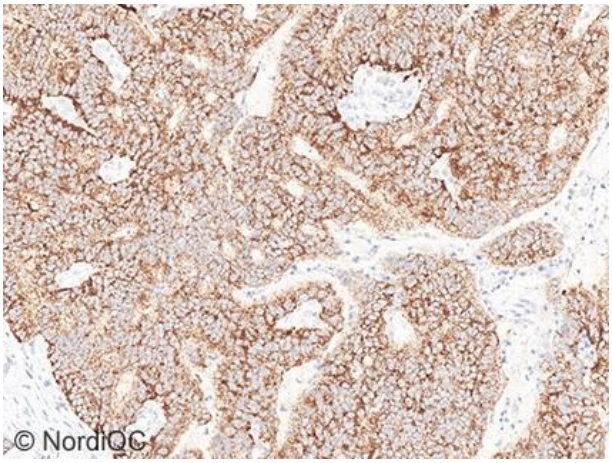


Fig. 5a
Optimal BRAF staining of the colon adenocarcinoma with BRAF V600E mutation, tissue core no. 5, using the Ventana RTU 760-5095 based on mAb clone VE1, using a modified protocol adding OptiView Amplification to the recommended protocol settings. The neoplastic cells show a strong cytoplasmic staining reaction.

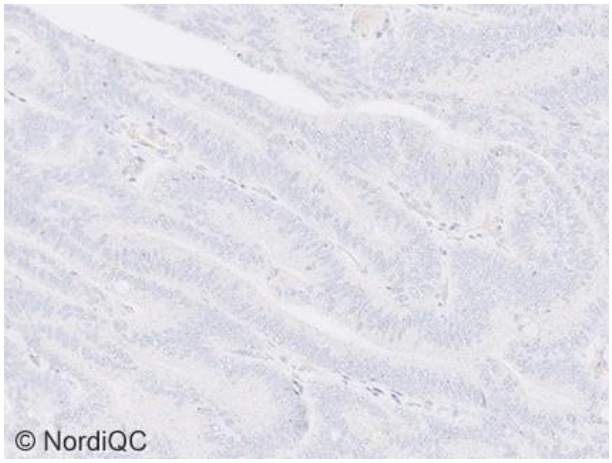


Fig. 5b
Optimal BRAF staining of the colon adenocarcinoma without BRAF V600E mutation, tissue core no. 4, using same protocol as in Fig. 5a. All cells are negative.

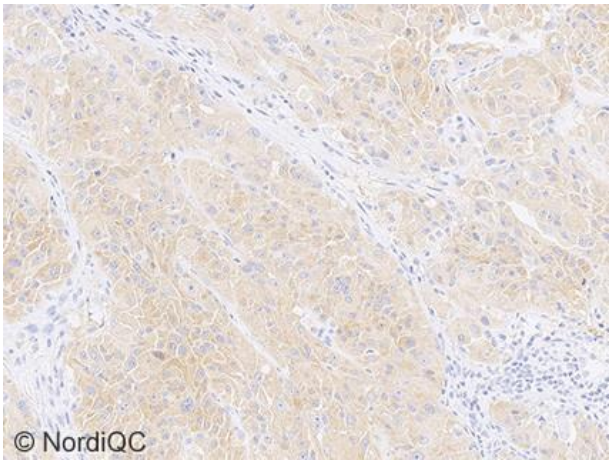


Fig. 6a
BRAF staining of the malignant melanoma with BRAF V600E mutation, tissue core no 1, using mAb clone VE1, diluted, 1:400, epitope retrieval using HIER in an alkaline buffer (40 min.) and a 3-step multimer based detection system
The neoplastic cells display a weak cytoplasmic staining reaction. Also compare with Fig. 6b, same protocol.

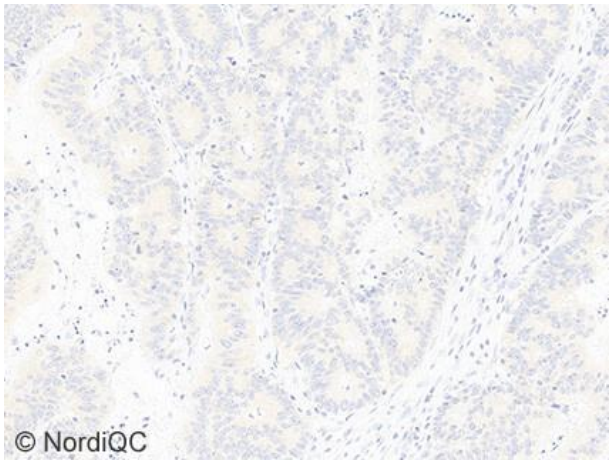


Fig. 6b
Insufficient BRAF staining of the colon adenocarcinoma with BRAF V600E mutation, tissue core no. 5, using same protocol as in Fig. 6a. The neoplastic cells show a too weak staining reaction and are scored as negative. Compare with the optimal result in Figs. 3a and 5a.

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