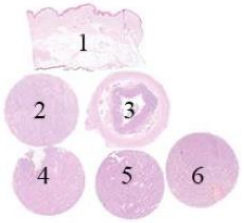
Assessment Run 60 2020

Transcription factor SOX-10 (SOX10)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for SOX10, identifying malignant melanomas and triple negative breast carcinoma in the characterization of tumours of unknown origin. Relevant clinical tissues, both normal and neoplastic, were selected displaying a broad spectrum of antigen densities for MLA (see below).

Material
The slide to be stained for SOX10 comprised:



1. Skin, 2. Colon adenocarcinoma, 3. Appendix, 4. Breast carcinoma, 5-6. Malignant melanoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a SOX10 staining as optimal included:

- A strong, distinct nuclear staining reaction of virtually all melanocytes in the skin and Schwann cells in the appendix.
- An at least moderate, distinct nuclear staining reaction of the majority of myoepithelial cells lining sweat glands in the skin.
- An at least moderate, distinct nuclear staining reaction of virtually all neoplastic cells in the breast carcinoma (triple negative).
- An at least moderate, distinct nuclear staining reaction of the majority of neoplastic cells in the malignant melanoma, tissue core no. 5.
- A strong, distinct nuclear staining reaction of the majority of neoplastic cells in the malignant melanoma, tissue core no. 6.
- No staining reaction in other cellular structures including the neoplastic cells of the colon adenocarcinoma.

A weak cytoplasmic staining reaction in cells with a strong nuclear staining reaction was accepted. For certain primary antibodies e.g. mAb clone EP268, a weak cytoplasmic staining reaction of ganglion/neuronic cells in the appendix was accepted, providing that interpretation of the specific nuclear staining reaction was not compromised.

Participation

Number of laboratories registered for SOX10, run 60	284
Number of laboratories returning slides	251 (88%)

Results
During the assessment a limited number of participants have experienced issues with the circulated NordiQC slides, providing a partial or entire aberrant/false negative staining result in some cases. During the assessment, this observation was taken into account and for SOX10, 1 slide was potentially affected and excluded. If performance was characterized by uneven staining or a completely false negative result that could be related to the quality of the slide and not the protocol submitted, this was commented in the individual assessment feed-back.
250 laboratories participated in this assessment. 230 (92%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks (see page 2).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Less successful primary antibody – especially polyclonal Abs (pAbs)
- Insufficient Heat Induced Epitope Retrieval (HIER) (too short heating time or HIER in acidic buffer).
- Less sensitive detection systems
- Unexplained technical issues

Performance history

This was the fourth NordiQC assessment of SOX10. The overall pass rate improved marginally compared to the result obtained in run 55, 2019 (see Table 2) and significantly compared to Runs 45 and 48.

Table 2. **Proportion of sufficient results for SOX10 in the four NordiQC runs performed**

	Run 45 2015	Run 48 2016	Run 55 2019	Run 60 2020
Participants, n=	86	120	204	250
Sufficient results	45%	68%	89%	92%

Conclusion

The mAb clones **BC34, BS7, ZM10**, and the rmAb clones **EP268** and **SP267** could all be used to obtain an optimal result for SOX10. Irrespective of the clone applied, efficient HIER (preferable in an alkaline buffer), a precise calibration of the primary Ab and the use of a 3-step multimer/polymer based detection system, were the main prerequisite for an optimal result. The RTU system 760-4968 (Ventana) based on the rmAb clone SP267, showed superior performance and following vendor recommended protocol settings, 98% (60/61) were assessed as sufficient of which 95% (58/61) were optimal.
Skin and colon/appendix are recommendable positive and negative tissue controls for SOX10. Virtually all melanocytes of the skin and Schwann cells of the appendix/colon must display a strong nuclear staining reaction, while the majority of myoepithelial cells in the sweat glands of the skin must show an at least moderate, but distinct nuclear staining reaction. No reactions should be seen in other cells.

Table 1. **Antibodies and assessment marks for SOX10, run 60**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **BC34**	42 3 3	Biocare Medical Abcam Zytomed Systems	16	26	4	2	88%	33%
mAb clone **BS7**	12	Nordic Biosite	6	6	0	0	100%	50%
mAb clone **ZM10**	2	Zeta Corporation	1	1	0	0		
mAb clone **SOX10/1074**	1	Immunologic	0	0	1	0	-	-
rmAb clone **EP268**	45 2 2 1 1	Cell Marque Epitomics BioSB Diagnostic Biosystems Diagomics	34	15	2	0	96%	67%
rmAb clone **SP267**	1 1	Spring Bioscience Abcam	1	1	0	0	-	-
pAb **10336**	1	ProteinTech	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **BC34 API 3099**[3]	1	Biocare Medical	1	0	0	0	-	-
mAb clone **BC34 API 3099**[4]	10	Biocare Medical	5	4	1	0	90%	50%
rmAb clone **SP267 760-4968**[3]	61	Ventana/Roche	58	2	1	0	98%	95%
rmAb clone **SP267 760-4968**[4]	36	Ventana/Roche	31	3	1	1	94%	86%
rmAb clone **EP268 383R**	17	Cell Marque	12	2	3	0	82%	71%
rmAb clone **EP268 MAD-000656QD**	1	Master Diagnostica	0	1	0	0	-	-
rmAb clone **EP268 PR135**	2	PathSitu Biotechnologies	1	1	0	0	-	-
rmAb clone **BP6024 I1015**	1	Tuling Biotechnology	1	0	0	0	-	-
pAb **383A-78**	4	Cell Marque	0	1	2	1	-	-
Total	250		167	63	16	4	-	
Proportion			67%	25%	6%	2%	92%	

1) Proportion of sufficient results (optimal or good). (≥5 asessed protocols).
2) Proportion of Optimal Results (OR).
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 applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 assessed protocols).

Detailed analysis of SOX10, Run 60
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **BC34**: Protocols with optimal results were all based on HIER using an alkaline buffer as Cell Conditioning 1 (CC1, Ventana) (7/20)*, Target Retrieval Solution (TRS) (3-in-1) pH 9 (Dako) (6/13) or Bond Epitope Retrieval Solution 2 (BERS2, Leica) (3/10) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 30 of 33 (91%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

mAb clone **BS7**: Protocols with optimal results were all based on HIER using an alkaline buffer as TRS (3-in-1) pH 9 (Dako) (4/6), CC1 (Ventana) (1/4) or Tris-EDTA/EGTA pH 9 (1/1). The mAb was typically diluted in the range of 1:100-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 7 of 7 (100%) laboratories produced a sufficient staining

mAb clone **ZM10**: One protocol with an optimal result was based on HIER using Tris-EDTA pH 9 (Zeta Corp.). The mAb was diluted 1:200 and Zeta Universal HRP Polymer/DAB was used as the detection system.

rmAb clone **EP268**: Protocols with optimal results were all based on HIER using either CC1 (Ventana) (14/20), TRS (3-in-1) pH 9 (Dako) (14/20), BERS2 (Leica) (5/8) or DBS Montage EDTA Antigen Retrieval Solution (Diagnostic Biosystem) (1/1) as retrieval buffer. The rmAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 34 of 36 (94 %) laboratories produced a sufficient staining.

rmAb clone **SP267**: One protocol with an optimal result was based on HIER using CC1 (Ventana). The rmAb was diluted 1:100 and OptiView (Ventana) was used as the detection system.

Table 3. **Proportion of optimal results for SOX10 for the most commonly used antibodies as concentrate on the four main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark 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 **BC34**	2/4**	-	4/8 (50%)	0/1	7/17 (41%)	0/0	3/8 (38%)	-
mAb clone **BS7**	1/1	-	3/4	-	0/3	-	-	-
rmAb clone **EP268**	0/1	-	9/10 (90%)	-	12/18 (67%)	-	3/5 (60%)	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 **BC34,** product no. **API 3099,** Biocare Medicare, IntelliPATH:
One protocol with an optimal result was based on HIER using Diva Decloaker (Pressure Cooker, efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH4 Universal HRP-polymer (M4U534, Biocare Medical) as the detection system.

rmAb clone **SP267,** product no. **760-4968,** Ventana/Roche Benchmark Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 32 min. incubation time of primary Ab, UltraView with or without amplification (760-500 + 760-080) or OptiView (760-700) as the detection system. Using these protocol settings, 74 of 75 (99%) laboratories produced a sufficient result.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). 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 SOX10 for the most commonly used RTU IHC system**

RTU system	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Biocare IntelliPATH mAb BC34 **API 3099**	1/1	1/1	1/1	1/1
VMS Ultra/XT/GX rmAb SP267 **760-4968**	98% (60/61)	95% (58/61)	94% (34/36)	86% (31/36)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Comments

In this assessment and in concordance with the observations in previous NordiQC assessments of SOX10, the prevalent feature of an insufficient staining reaction was a too weak or false negative staining reaction of cells expected to be demonstrated and was seen in 60% (12/ 20) of the insufficient results. The majority of the laboratories were able to stain for SOX10 in Schwann cells of the appendix, the neoplastic cells of the melanoma tissue core no. 6, whereas demonstration of SOX10 in the neoplastic cells of the melanoma tissue core no. 5, the neoplastic cells of the breast carcinoma, normal melanocytes and myoepithelial cells of the skin was more challenging and required a carefully calibrated protocol. In 40% (8/20) of the insufficient results, a general poor signal-to-noise ratio and/or false positive staining reaction was seen.

In this assessment the pass rate was high and grouped together, both for laboratory developed (LD) and Ready-to-Use (RTU) assays, 92% (230/250) of the participants produced a sufficient result. Thus, any firm conclusion in regard of causes to insufficient staining results for both concentrated and RTU formats is difficult to elucidate upon due to the limited amount of data. However, the most frequent causes for insufficient results observed was use of protocol settings giving a low analytical sensitivity often applied in combinations as HIER in a citric based buffer and/or too diluted primary Ab and/or a 2-step polymer/multimer detection system.
The mAb clone BC34 and the rmAb clone EP268 were the most widely used antibodies within a LD assay. The mAb clone BC34 gave an overall pass rate of 88% (42/48). However, only 33% (16/48) was optimal (see Table 1). As shown in Table 3, optimal results could be obtained on all automated and semi-automated IHC platforms from Dako, Leica and Ventana. Protocols providing an optimal result were based on efficient HIER in an alkaline buffer e.g. BERS2 (Leica) or TRS pH 9 (Dako) with an average HIER time of 39 min. (range 20-64min.), an average dilution factor of 1:53 (range 1:25-1:100) and virtually all laboratories (15/16) applied a 3-step polymer/multimer detection system e.g. Bond refine (Leica) or Envision Flex+(Dako). For the mAb clone BC34, the protocol settings listed above are the basic requirements for optimal performance and demonstration of SOX10 in structures with both low-level and high-level SOX10 expression, which is the range seen in e.g. both malignant melanomas and triple negative breast carcinomas.

The LD assays based on rmAB clone EP268 provided a pass rate of 96% (49/51) of which 67% (34/51) were assessed as optimal. The prerequisites for obtaining an optimal result were the use of efficient HIER in alkaline buffer with an average HIER time of 36 min. (range 20-88 min.), an average dilution factor of 1:182 (range 1:50-1:400) and as for the mAb clone BC34, virtually all laboratories (30/31) used a 3- step 3-step polymer/multimer based detection system. The two protocols assessed as insufficient applied similar protocol settings regarding HIER and Ab titre, but both laboratories used a 2-step multimer detection system (Ultraview, Ventana) as part of the protocol settings.

Twelve laboratories used the mAb clone BS7 within a LD assay and all (12/12) were assessed as sufficient. 50% (6/12) of the protocols were assessed as optimal. The results marked as good were typically characterized by reduced signal-to-noise ratio and/or a reduced intensity/proportion of cells demonstrated. Data from internal NordiQC reference laboratories have revealed that the clone is highly recommendable but can be challenging to calibrate accurately to find right level of analytical sensitivity versus unwanted background reaction.

In this assessment, the RTU system 760-4968 (Ventana) based on the rmAb clone SP267 was the most widely used assay for the demonstration of SOX10. The number of participants using this system, has increased significantly during the last three runs; 48 (2016), 55 (2019) and 60 (2020) - from 5 to 97 laboratories. The assays based on this system have shown to be very robust, and over the last three runs, the accumulated pass rate has been 98% (163/166) of which 92% (152/166) assessed as optimal. As shown in Table 4, and for this run 60, the highest proportion of sufficient and optimal results were obtained using the RTU system according to the protocol recommendations provided by the vendor. For

laboratories applying OptiView as the detection system, 94% (33/35) of the slides were assessed as optimal. One laboratory obtained an insufficient result most likely related to unidentified technical issues. Using UltraView as the detection system, and following vendor recommended protocol settings based on HIER in CC1 (64min.) and incubation in primary Ab for 32 min., the proportion of sufficient results was 100% (26/26) of which 96% (25/26) were optimal, emphasizing that the assay is very robust and less sensitive protocol settings can be applied for optimal performance. Laboratory modified protocol settings (typically adjusting HIER and incubation time of the primary Ab) could also provide optimal results, but proportion of optimal results decreased from 95% to 86% compared to vendor recommended protocol settings.

One laboratory obtaining an optimal result, used the RTU system API 3099 based on the mAb clone BC34 (Biocare Medical) developed for the IHC stainer IntelliPATH (see protocol settings above). However, eight laboratories used the RTU format on platforms other than the IntelliPATH (Biocare). Off-label use of an RTU format, validated for a given IHC system e.g. platform including immuno-reagents, is not recommended despite obtaining a relative high pass rate (see Table 1). Essentially, a RTU format of a primary Ab is used within a system with precise information on vendor recommended protocol settings, equipment, reagents and results expected. This "inappropriate/incorrect" use of an RTU product was also seen with other RTU formats e.g. 383R based on the rmAb clone EP268 from Cell Marque. Overall the off-label use of the RTU formats API 3099 and 383R on non-compliant platforms gave a relatively high pass rate but reduced proportion of optimal results to e.g. the Ventana RTU system.

This was the fourth assessment of SOX10 in NordiQC (see Table 2). The pass rate increased marginally compared to the latest run 55, 2019. Several parameters contributed to the high proportion of sufficient results: 1) The extended use of robust primary Abs (e.g. EP268), 2) The superior performance of the RTU system 760-4968 (Ventana) based on the rmAb clone SP267 and applied by 39% (97/250) of the participants, 3) The number of laboratories using pAbs, providing consistent poor results, is still at a low level - 2% (5/250) in this assessment, 4) Laboratories following information giving by the NordiQC organization in past runs, typical providing specific recommendations to use HIER in an alkaline buffer, careful calibration of the primary Ab and the use of an 3-step multimer/polymer detection system. Importantly, the protocols must give staining results accordingly to the expected pattern and antigen level within the recommended tissue control material (see below). This seems highly beneficial and central for both the initial validation process and verification of IHC test reproducibility for SOX10.

Controls

Skin and colon/appendix are recommended as positive and negative tissue controls for SOX10. In skin, strong nuclear staining reaction in virtually all melanocytes must be seen. The vast majority of myoepithelial cells lining sweat glands must show an at least moderate nuclear staining reaction. In colon/appendix, virtually all Schwann cells must display an as strong as possible nuclear staining reaction without any staining reaction of epithelial cells and e.g. smooth muscle cells. At present, and as specified in previous assessments, no reliable tissue component with consistent low level expression of SOX10 has been identified, monitoring the reproducibility and overall analytical sensitivity of the assay. Thus, both skin and colon/appendix are needed as tissue controls for SOX10.

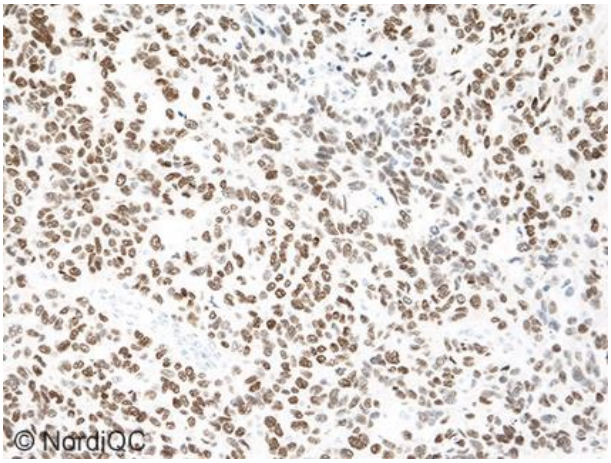


Fig. 1a (x200)
Optimal SOX10 staining of the melanoma, tissue core no.5, using the rmAb SP267 (RTU system 760-4968, Ventana) following recommendations given by the vendor - HIER in CC1 (64 min.), 32 min. incubation time in primary Ab and UltraView (Ventana) as detection system – same protocol used in Figs. 2a-6a. Virtually all the neoplastic cells show an at least moderate but distinct nuclear staining reaction.

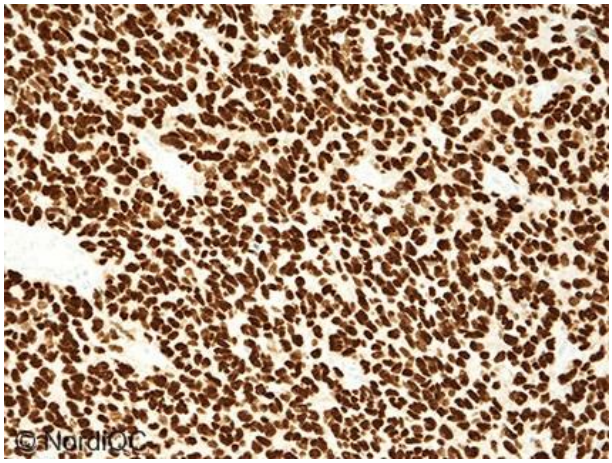


Fig. 1b (x200)
SOX10 staining of the melanoma, tissue core no.5, using the rmAb SP267 (RTU system 760-4968, Ventana) applying laboratory modified protocol settings as HIER in CC1 (80 min.), 24 min. incubation time in primary Ab and OptiView with amplification (Ventana) as detection system – same protocol used in Figs. 2b-6b. Although the nuclei of the neoplastic cells display strong staining intensity, the assay was overall challenged by poor signal-to-noise ration/false positive staining reaction and impaired morphology due to excessive antigen retrieval - compare with Figs. 1a-6b. The result overall assessed as insufficient.

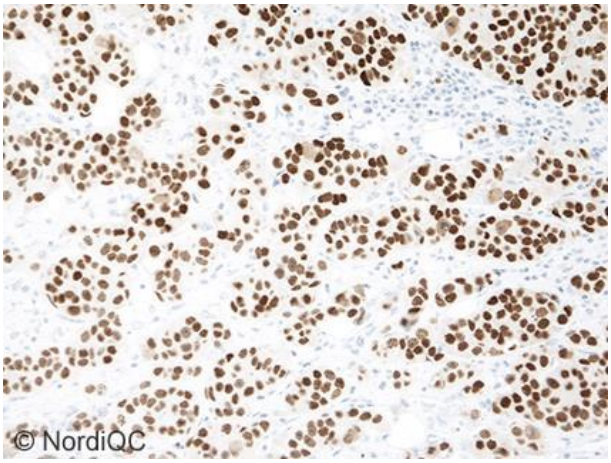


Fig. 2a (x200)
Optimal SOX10 staining of the breast carcinoma using same protocol as in Fig. 1a. Virtually all neoplastic cells show a distinct, moderate to strong nuclear staining reaction. No background staining is seen. The RTU system is well calibrated and it is advisable to follow protocol recommendations giving by the vendor, both for protocols based on UltraView and OptiView as the basic detection system. There is no need to enhance sensitivity further, risking problems as displayed in Fig. 4b-6b.

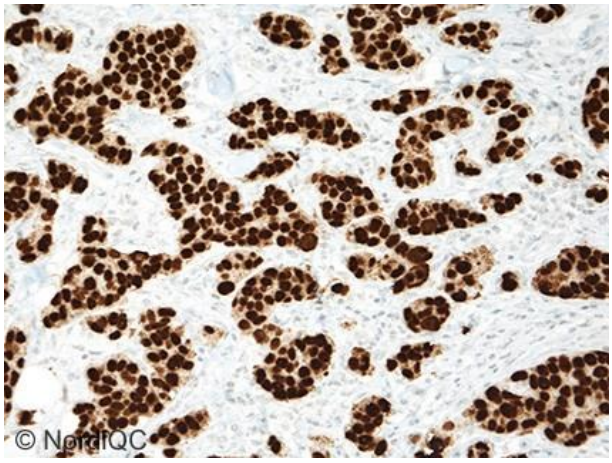


Fig. 2b (x200)
SOX10 of breast carcinoma using same protocol as in Fig. 1b. The staining intensity of the neoplastic cells is strong, but due to protocol settings based on a very high analytical sensitivity, a weak background staining is displayed. The problem is in particular shown in Fig. 4b-6b. Compare with Fig. 2a.
The result overall assessed as insufficient.

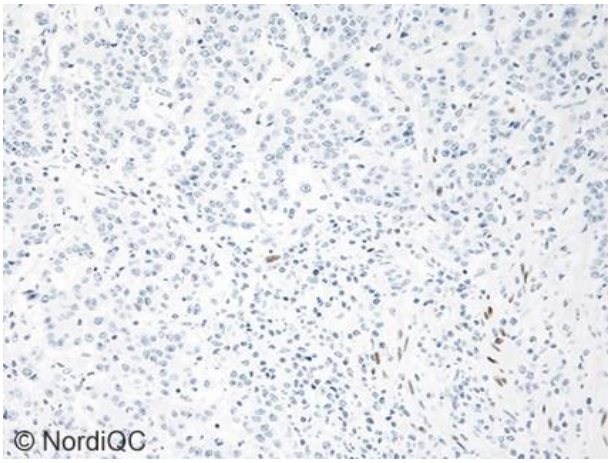


Fig. 3a (x200)
Optimal SOX10 staining of the colon adenocarcinoma using same protocol as in Figs. 1a-2a. The neoplastic cells are negative as expected. Only scattered neurons/Schwann cells intermingling with the neoplastic cells are positive.

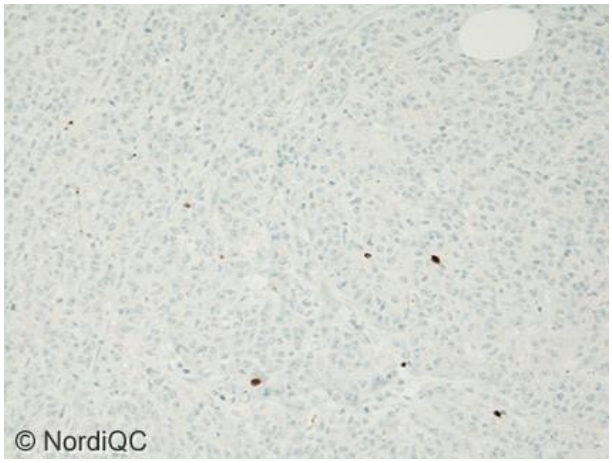


Fig. 3b (x200)
SOX10 staining of the colon adenocarcinoma using same protocol as in Figs. 1b-2b. The neoplastic cells show the expected staining pattern. However, the protocol applied is unreliable due to protocol settings based on too high analytical sensitivity, risking false positive staining reactions. The problem is in particular shown in Fig. 4b-6b.
The result overall assessed as insufficient.

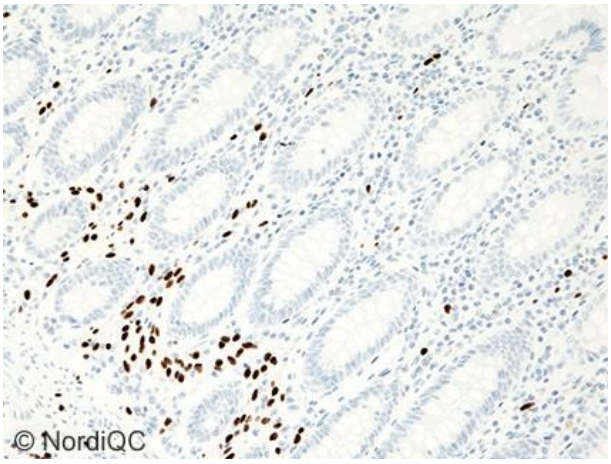


Fig. 4a (x200)
Optimal SOX10 staining of the appendix using same protocol as in Figs. 1a-3a. Virtually all Schwann cells in lamina propria mucosa show a strong nuclear staining reaction. The epithelial and smooth muscle cells are negative.

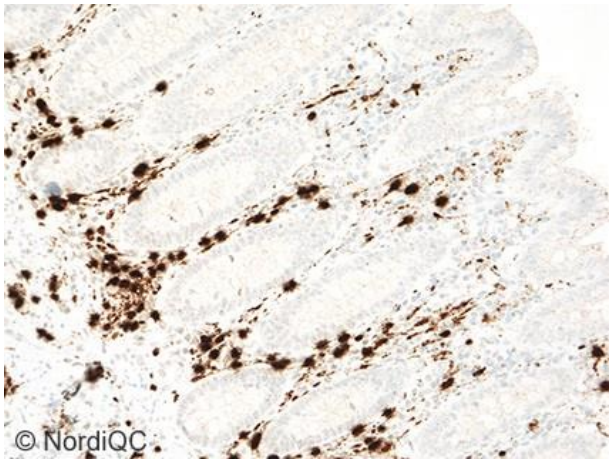


Fig. 4b (x200)
Insufficient SOX10 staining of the appendix using same protocol as in Figs. 1b-3b. The staining intensity is enhanced but the reaction is imprecise, displaying an indistinct signal in especially the nuclei of Schwann cells (most likely related to excessive HIER). In addition, an aberrant cytoplasmic granular staining reaction is seen in both stromal and epithelial cells, a problem often related to the use of tyramide based detection systems as OptiView with amplification - compare with Fig. 4a.

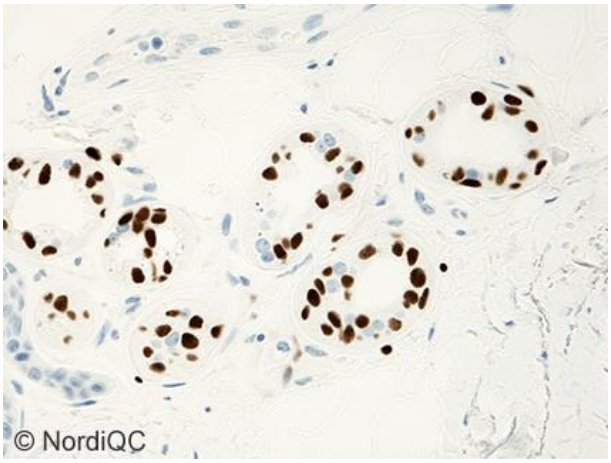


Fig. 5a (x400)
Optimal SOX10 staining of the skin using same protocol as in Figs. 1a-4a. Virtually all the myoepithelial cells of the sweat glands show an at least moderate, distinct nuclear staining reaction. An optimal balance between luminal and myoepithelial cells is obtained.

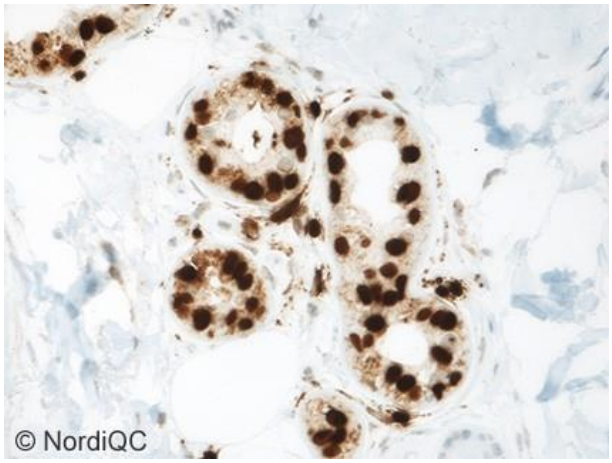


Fig. 5b (x400)
Insufficient SOX10 staining of the skin using same protocol as in Figs. 1b-4b. The morphology is impaired, the staining reaction is indistinct which includes a cytoplasmic granular deposition of the chromogen, providing a suboptimal balance between luminal and myoepithelial cells, making it difficult to discriminate these two cells types from each other - compare with Fig. 5a.

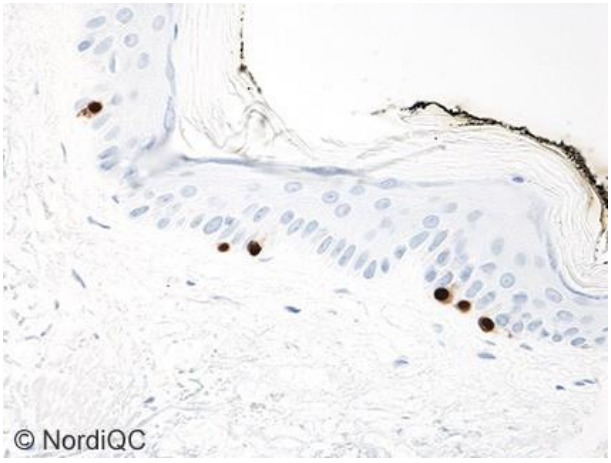


Fig. 6a (x400)
Optimal SOX10 staining of the skin using same protocol as in Figs. 1a-5a. All melanocytes show a strong, distinct nuclear staining reaction. No background is seen.

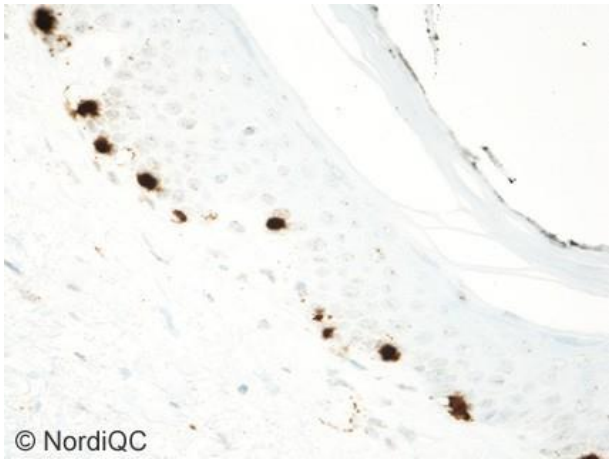


Fig. 6b (x400)
Insufficient SOX10 staining of the skin using same protocol as in Figs. 1b-5b. Although the melanocytes are positive, the morphological details are impaired, and an aberrant cytoplasmic granular staining reaction is seen in stromal and squamous epithelial cells - compare with Fig. 6a.

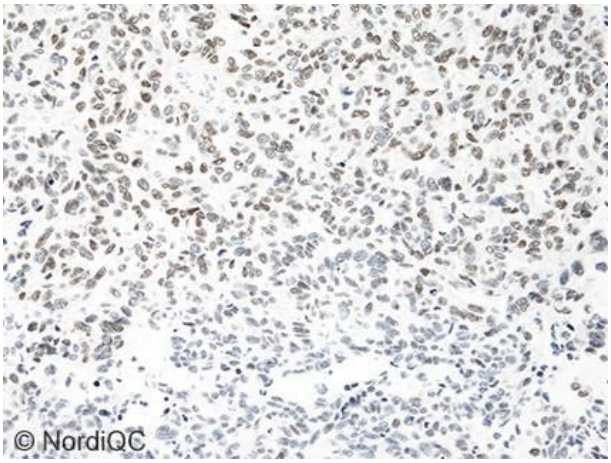


Fig. 7a (x200)
Insufficient SOX10 staining of the melanoma, tissue core no. 5, using a protocol with too low analytical sensitivity; HIER in acidic buffer (TRS pH 6, Dako), too diluted primary Ab (mAb BC34) and the 2-step polymer Envision Flex (Dako) as the detection system – same protocol used in Fig. 7b. The nuclei of the neoplastic cells display a reduced intensity and also the proportion of positive cells is reduced – compare with Fig. 1a. The use of low sensitive protocol settings, often in combination, were frequently seen in protocols assessed as insufficient.

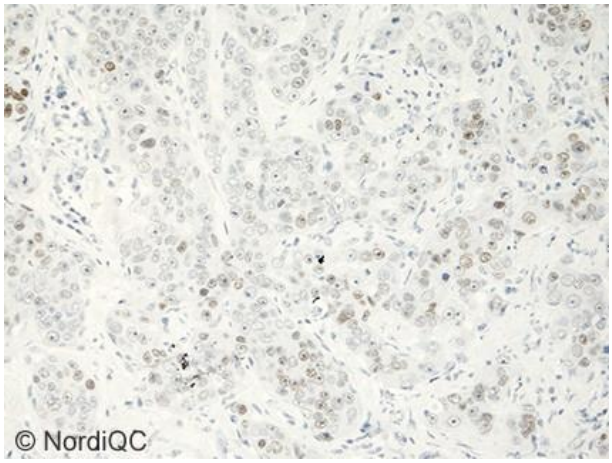


Fig. 7b (x200)
Insufficient SOX10 staining of the breast carcinoma using the same protocol as in Fig. 7a. Virtually all nuclei of the neoplastic cells show reduced staining intensity or being completely false negative.

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