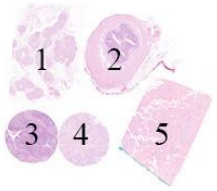
Assessment Run 66 2022

SMH (Myosin, smooth muscle heavy chain)

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for SMH, typically used in the diagnostic work-up in breast samples to distinguish benign and precursor lesions from invasive cancer. Relevant clinical tissues, both normal and neoplastic, were selected to display a broad spectrum of antigen densities for SMH (see below).

Material
The slide to be stained for SMH comprised:



1: Breast ductal carcinoma in-situ (DCIS), 2: Appendix, 3: Tonsil, 4: Breast ductal carcinoma, 5: Breast hyperplasia.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing SMH staining as optimal included:

- A moderate to strong and distinct cytoplasmic staining reaction of virtually all vascular smooth muscle cells in all specimens.
- An at least weak but distinct cytoplasmic staining of the vast majority of follicular dendritic cells in the tonsillar germinal centers.
- A moderate to strong, distinct cytoplasmic staining reaction of myoepithelial cells lining ductal glands in the breast hyperplasia and in remnants of normal glands in the breast DCIS and breast ductal carcinoma.
- A moderate to strong, distinct cytoplasmic staining reaction of myoepithelial cells lining DCIS components in the breast DCIS and breast ductal carcinoma.
- No staining of epithelial cells in the breast specimens, appendix and tonsil.

Participation

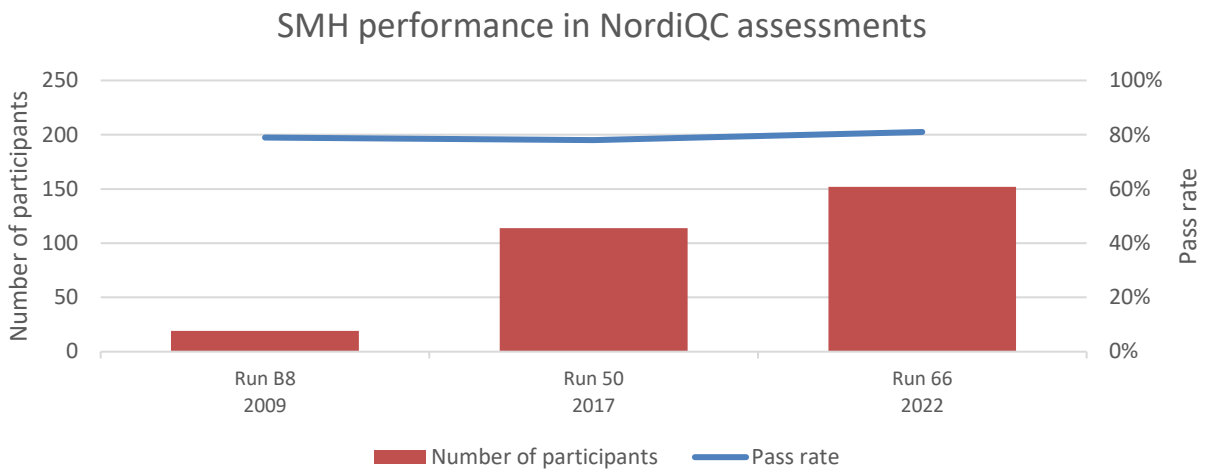
Number of laboratories registered for SMH, run 66	183
Number of laboratories returning slides	163 (89%)

Results
163 laboratories participated in this assessment. 11 participants used an inappropriate antibody. These participants were not included in the analysis. Of the remaining 152 laboratories, 81% achieved a sufficient mark (optimal or good).
Table 1 summarizes the antibodies (Abs) used and assessment marks given (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 reactions were:
- Off-label use of RTU formats
- Use of less successful Abs
- Less sensitive detection systems

Performance history
This was the third NordiQC assessment of SMH.
Virtually the same pass rate has been seen in the three runs performed (see Graph 1).

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



Conclusion
The mAb clones **SMMS-1** and **S131** were the two most widely used and successful antibodies for SMH. mAb clone SMMS-1 was used by the majority of laboratories and optimal results were obtained both within a laboratory developed (LD) assay on the main IHC platforms and as a Ready-To-Use system (Dako/Agilent and Ventana/Roche). Within a LD assay, efficient HIER in an alkaline buffer and use of a sensitive and specific 3-step polymer / multimer based detection system gave the highest proportion of sufficient and optimal results for mAb clone SMMS-1.
The Leica Biosystems Ready-To-Use system based on mAb clone S131 was most successful and applied by the vendor recommended protocol a pass rate of 100%, all optimal, was observed.

Controls
Tonsil was found to be the preferred positive and negative tissue control for SMH. Virtually all smooth muscle cells in vessels must show a moderate to strong cytoplasmic staining reaction, while the follicular dendritic cells in the germinal centres must show an at least weak to moderate but distinct staining reaction.
No staining should be seen in lymphocytes and epithelial cells.

Table 1. **Antibodies and assessment marks for SMH, run 66**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **SMMS-1**	12 18 3 2 1	Dako/Agilent Cell Marque Biocare Medical Zytomed Systems Diagnostic BioSytems	25	6	4	1	86%	69%
rmAb clone **EPR5336**	1	Abcam	1	0	0	0	-	-
rmAb clone **BP6091**	1	Biolynx	1	0	0	0	-	-
rmAb clone **EP166**	3	Cell Marque	1	0	1	1	-	-
Ready-To-Use antibodies								
mAb clone **S131 PA0493**[3]	7	Leica Biosystems	7	0	0	0	100%	100%
mAb clone **S131 PA0493**[4]	3	Leica Biosystems	2	1	0	0	-	-
mAb clone **SMMS-1 760-2704**[3]	15	Ventana/Roche	10	3	2	0	87%	67%
mAb clone **SMMS-1 760-2704**[4]	40	Ventana/Roche	24	14	2	0	95%	60%
mAb clone **SMMS-1 IR066/IS066**[3]	11	Dako/Agilent	2	6	3	0	73%	18%
mAb clone **SMMS-1 IR066/IS066**[4]	24	Dako/Agilent	6	5	7	6	46%	25%
mAb clone **SMMS-1 298M-18**	2	Cell Marque	2	0	0	0	-	-
mAb clones **SMMS-1 PM420AA**	1	Biocare	1	0	0	0	-	-
mAb clone **SMMS-1 8517-C010**	2	Sakura Finetek	2	0	0	0	-	-
mAb clone **SMMS-1 CMM-0360**	1	Celnovte	1	0	0	0	-	-
mAb clone **IHC091 IHC091**	1	GenomeMe	1	0	0	0	-	-
Ab clone **MX109 MAB-1010**	1	Maixin	1	0	0	0	-	-
Ab clone **614G0B2 PA443**	1	Abcarta	0	0	1	0	-	-
rmAb clone **MYH11/4337R ANC62-5M**	1	BioGenex	0	0	0	1	-	-
rmAb clone **EP166 MAD-000718QB**	1	Master Diagnostica	1	0	0	0	-	-
Total	152		88	35	20	9		
Proportion			58%	23%	13%	6%	81%	

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).

Detailed analysis of SMH, Run 66
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **SMMS-1**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) High pH (Dako/Agilent) (6/8)*, Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (6/8), Bond Epitope Retrieval Solution 1 (BERS1, Leica Biosystems) (1/2), Cell Conditioning 1 (CC1, Ventana/Roche) (8/12), Tris-EDTA pH 9 (1/1), EDTA pH 8 (1/1) or Citrate buffer pH 6 (1/1) as retrieval buffer. Two optimal protocols were based on a combined pre-treatment with HIER in CC1 followed by proteolysis in Protease 3 (Ventana/Roche). The mAb was typically diluted in the range of 1:75-1:1,000, depending on the total sensitivity of the protocol employed. Using these protocol settings, 31 of 35 (89%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for SMH for the most commonly used antibody as concentrate on the 4 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 **SMMS-1**	0/1**	-	6/7 (86%)	0/1	8/12 (67%)	-	6/8 (75%)	(1/1)

* 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 **S131** product no. **PA0493**, Leica Biosystems, BOND III / BOND MAX:
Protocols with optimal results were based on HIER using BERS2 (efficient heating time 20 min. at 99-100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 10 of 10 (100%) laboratories produced a sufficient staining result (optimal or good).

mAb clone **SMMS-1**, product no. **760-2704**, Ventana/Roche, BenchMark XT / Benchmark Ultra:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min.), and 12-32 min. incubation of the primary Ab. UltraView (760-500) +/- amplification kit or OptiView (760-700) were used as detection systems. Using these protocol settings, 48 of 51 (94%) laboratories produced a sufficient staining result.

mAb clone **SMMS-1** product no. **IR066/IS066**, Dako/Agilent, Autostainer+ / Autostainer Link:
Protocols with optimal results were based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 20-30 min. at 95-99°C), 10-20 min. incubation of the primary Ab and EnVision FLEX or FLEX+ (K8000 -/+ K8002) as detection systems. Using these protocol settings, 13 of 17 (76%) laboratories produced a sufficient staining result.

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 specific IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for SMH for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb SMMS-1 **IR/IS066**	73% (8/11)	18% (2/11)	86% (6/7)	57% (4/7)
Leica BOND mAb S131 **PA0493**	100% (7/7)	100% (7/7)	3/3	2/3
VMS Ultra/XT mAb SMMS-1 **760-2704**	87% (13/15)	67% (10/15)	95% (38/40)	60% (24/40)

* 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 concordance with the previous NordiQC assessments for SMH, the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 73% of the insufficient results (21/29). 10% of the insufficient results were caused by a false positive staining reaction (3/29) The remaining 17% of the insufficient results were characterized by a generally poor signal-to-noise ratio and/or excessive background reaction (5/29). A too weak staining reaction was typically characterized by a reduced intensity and proportion of cells expected to be demonstrated. This was in particular observed in the tonsillar follicular dendritic cells and the myoepithelial cells of both normal breast glands and lining the DCIS components. Virtually all laboratories successfully demonstrated SMH in smooth muscle cells of large vessels and lamina muscularis (appendix).

27% (41 of 152) of the laboratories used concentrated Ab formats within LD assays for SMH. The mAb clone SMMS-1 was the most widely used Ab and could be used to obtain optimal staining results on all fully automated IHC platforms as shown in Table 2. Used within a LD assay, the mAb clone SMMS-1 gave an overall pass rate of 86% and 69% of the results were assessed as optimal (see Table 1).
Efficient HIER in an alkaline buffer either as single retrieval method or in combination with mild proteolytic pre-treatment was found essential for optimal performance. HIER in low pH buffers or proteolysis as single retrieval method was found to be less successful.

73% (111 of 152) of the laboratories used Abs in Ready-To-Use (RTU) formats. The most frequently used RTU systems for SMH were the Ventana/Roche 760-2704 system for BenchMark and the Dako/Agilent IR066/IS066 system for Autostainer, both based on mAb clone SMMS-1.
The Ventana/Roche RTU system was the most widely used RTU system applied by 55 laboratories. An overall pass rate of 93% was seen, 62% optimal. Optimal results could be achieved both by the vendor recommended protocol settings and by laboratory modified protocol settings (see Table 3). Most common modifications were prolonging HIER and/or incubation time of primary Ab and use of OptiView as detection system instead of UltraView.

The Dako/Agilent IR066/IS066 system for Autostainer provided sufficient and optimal results both by vendor recommended and laboratory modified protocol settings. Used accordingly to the recommended protocol settings on Autostainer, a pass rate of 73%, 18% optimal was obtained (see Table 3). When the IR066/IS066 system was applied on Autostainer by laboratory modified protocol settings, an improved pass rate at 86%, 57% optimal was observed. The most common and successful modifications were based on prolonging HIER and/or incubation time of the primary Ab. Lacking a RTU alternative tailored for the Dako Omnis platform, 13 laboratories used the IR066/IS066 system on the Dako Omnis giving a pass rate at 15% (2 of 13), no optimal results were obtained. All the insufficient results were commented as weak, false negative or poor-signal-to-noise-ratio characterized by extensive background reaction combined with difficulties to demonstrate low-level antigen expressing structures. In this context, it was more successful to use the concentrated format of mAb clone SMMS-1 on Dako Omnis (see Table 2) as alternative to the off-label use of a RTU format not being developed and validated for the respective IHC platform.

The Leica Biosystems RTU system PA0493 based on mAb clone S131 was used by 10 participants, and all produced a sufficient result, 90% optimal.
Laboratories applying the vendor recommended protocol settings, using HIER in BERS2 for 20 min., 15 min. incubation of the primary Ab and Refine as detection system all obtained optimal results (see Table 3).

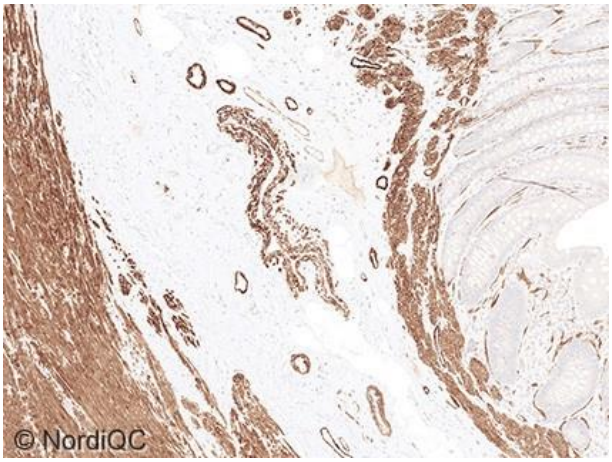


Fig. 1a
Optimal staining reaction for SMH of the appendix using the mAb clone SMMS-1 RTU (Ventana/Roche, 760-2704) within laboratory modified protocol settings, using OptiView as detection system.
Virtually all smooth muscle cells in vessels and lamina muscularis show a moderate to strong cytoplasmic staining reaction.
Also compare with Figs. 2a – 5a, same protocol.

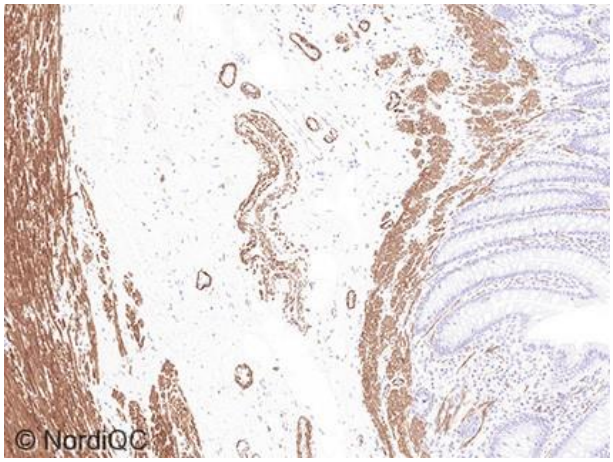


Fig. 1b
SMH staining reaction of the appendix using an insufficient protocol based on the mAb clone SMMS-1 RTU (Dako/Agilent, IR066) developed for the Autostainer, but applied on the Dako Omnis platform.
In appendix – same field as Fig. 1a, a weak to strong staining reaction is seen in virtually all smooth muscle cells as expected.
Also compare with Figs. 2b – 5b, same protocol.

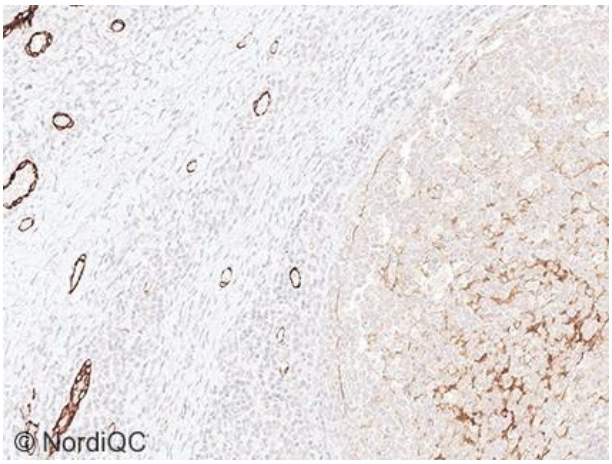


Fig. 2a
Optimal SMH staining reaction of the tonsil using same protocol as in Fig. 1a.
A weak to moderate staining reaction is seen in the follicular dendritic network in the germinal center.
A high signal-to-noise ratio is observed.

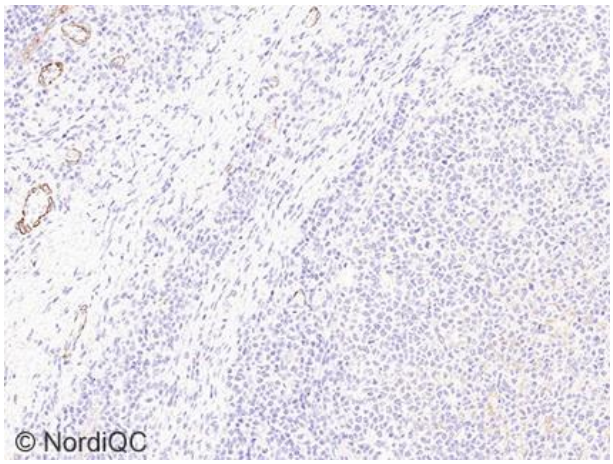


Fig. 2b
Insufficient SMH staining reaction of the tonsil using same protocol as in Fig. 1b - same field as Fig. 2a.
The follicular dendritic network in the germinal center is virtually negative and only the smooth muscle cells of large vessels are demonstrated.

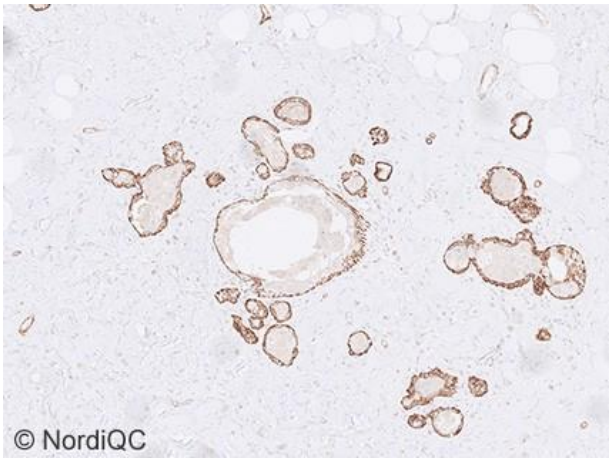


Fig. 3a
Optimal SMH staining reaction of the breast hyperplasia using same protocol as in Figs. 1a and 2a.
A moderate and distinct staining reaction is seen in virtually all myoepithelial cells lining the breast glands.

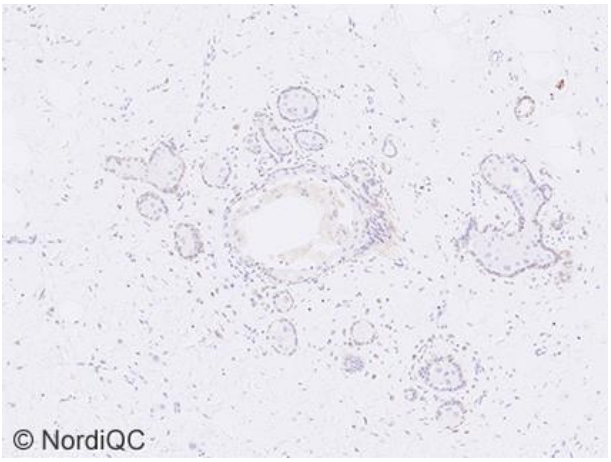


Fig. 3b
Insufficient SMH staining reaction of the breast hyperplasia using same protocol as in Figs. 1b and 2b – same field as Fig. 3a.
Only a faint staining reaction is seen in few myoepithelial cells lining the breast glands.

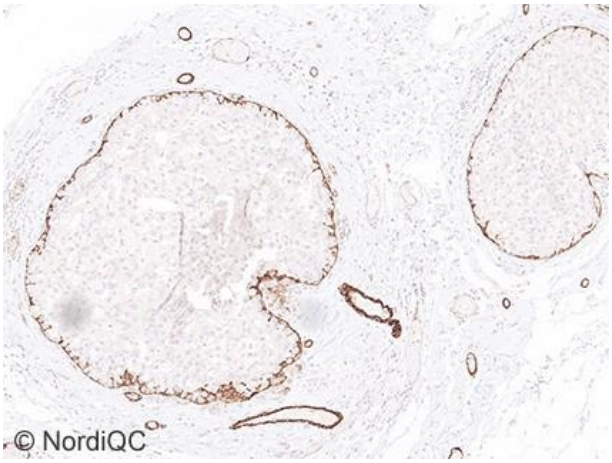


Fig. 4a
Optimal SMH staining reaction of the breast DCIS using same protocol as in Figs. 1a - 3a.
A moderate, distinct and continuous staining reaction is seen in the myoepithelial cells lining the breast DCIS component.

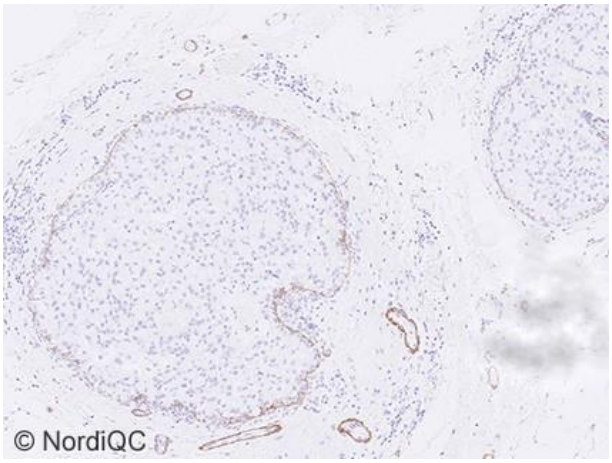


Fig. 4b
Insufficient SMH staining reaction of the breast DCIS using same protocol as in Figs. 1b – 3b – same field as Fig. 4a.
Only a weak and disrupted staining reaction is seen in the myoepithelial cells lining the breast DCIS component.

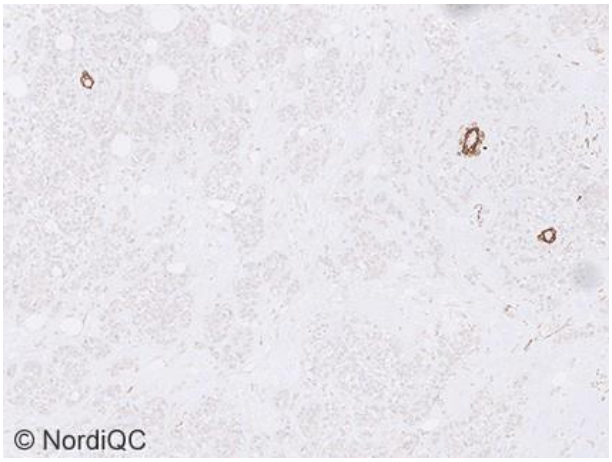


Fig. 5a
Optimal SMH staining reaction of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
The invasive components show no staining. A moderate staining reaction is seen in myoepithelial cells of entrapped normal glands.

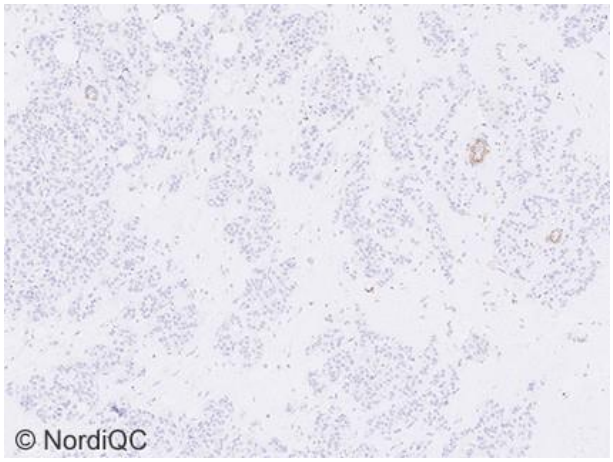


Fig. 5b
Insufficient SMH staining reaction of the breast ductal carcinoma using same protocol as in Figs. 1a - 4a.
No staining is seen in the invasive components, however, only a weak staining reaction is observed in the myoepithelial cells of normal glands.

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